Aus der Klinik für Unfall- und Wiederherstellungschirurgie
Charité – Universitätsmedizin Berlin
Direktor: Universitätsprofessor Dr. med. Norbert P. Haas

EINFLUSS VON CAGEDESIGN, CARRIER-SYSTEMEN UND WACHSTUMSFAKTOREN AUF DIE INTERVERTEBRALE SPONDYLODESE

Biomechanische und tierexperimentelle Untersuchungen an der Halswirbelsäule des Schafs

HABILITATIONSSCHRIFT

zur Erlangung der venia legendi für das Fach Chirurgie
vorgelegt der medizinischen Fakultät Charité der Humboldt-Universität zu Berlin
von

Dr. med. Frank Kandziora
geboren in Donauwörth

Präsident: Prof. Dr. rer. nat. J. Mlynek
Dekan: Prof. Dr. J. W. Dudenhausen

Gutachter: Prof. Dr. W. Dick
Prof. Dr. M.Blauth

Datum des Vortrages vor dem Fakultätsrat: 23.10.2003

Meiner geliebten Frau und meiner Mutter gewidmet.

„Die Forschung ist immer auf dem Wege, nie am Ziel.“

Adolf Pichler, Ritter von Rautenkar, 1819-1900

Schriftsteller und Naturwissenschaftler

1 Einleitung

1.1 Ventrale intervertebrale Spondylodese

Klinische Erfahrungen zeigen, dass es bei traumatischen und degenerativen Diskopathien der mittleren und unteren Halswirbelsäule zu einschneidenden gesundheitlichen Beeinträchtigungen und sozioökonomischen Problemen für die Patienten kommen kann. Seit der Einführung des ventralen Zugangs zur Halswirbelsäule durch Robinson [159] und der ventralen intervertebralen Spondylodese durch Smith-Robinson [3] und Cloward [3,31] werden weltweit zervikale Diskopathien mit gutem klinischen Erfolg operativ behandelt. Die anteriore zervikale Diskektomie erlaubt eine sichere Dekompression des Spinalkanals und der Neuroforamina, mit einer Linderung der klinischen Symptome Schmerz, Radikulopathie und Myelopathie [7,15,31,32,50,159,158,168]. Alleinige zervikale Diskektomien, ohne anschließende Rekonstruktion des sagitalen Wirbelsäulenprofils durch eine intervertebrale Stabilisierung, können zu einem Kollaps des Bandscheibenraums mit kyphotischer Fehlstellung im betroffenen Segment und in der Folge zu rezidivierenden klinischen und neurologischen Beschwerden führen [15,20,50,116,112,146,159,158,168]. Daher wird nach Diskektomie allgemein eine ventrale intervertebrale Spondylodese des betroffenen Segmentes unter Erhalt der Bandscheibenraumhöhe empfohlen [3,15,20,20,50,44,146,159,158].

1.1.1 Autologes Knochenmaterial

Der autologe trikortikale Beckenkammspan war jahrzehntelang das Implantat der Wahl zur ventralen interkorporellen Spondylodese der Halswirbelsäule [32,44,50,159,158]. Jedoch steht das autologe Knochenmaterial nur in begrenzter Menge zur Verfügung, was speziell bei multisegmentalen Fusionen von Bedeutung sein kann. Zusätzlich ist die operative Entnahme des trikortikalen Beckenkammspanimplantates und der Beckenkammspongiosa mit einer nicht unerheblichen Entnahmemorbidität assoziiert [4,8,60]. In zahlreichen Studien wurde dabei in Abhängigkeit vom Ausbildungsstand des Operateurs, der Operationstechnik, dem Evaluationszeitpunkt und den Evaluationskriterien die Anzahl der betroffenen Patienten zwischen 0,7 % und 75 % angegeben [4,8,10,35,52,60,75,150, 169,168,173,183,189,205]. Auch die Menge und Art des entnommenen

Knochenmaterials spielen dabei eine entscheidende Rolle. Die Entnahme eines trikortikalen Beckenkammspans ist mit einer höheren Entnahmemorbidität assoziiert als die Entnahme von autologer Beckenkammspongiosa [35,60,75]. Als potentielle Folgen der operativen Gewinnung autologen Knochenmaterials am Beckenkamm wurden Schmerzen an der Entnahmestelle [8,10,35,60,75,169,168,173,183,189], Hämatome [168,169], Wundinfektionen [173], Wundheilungsstörungen [169], Narbenbildungen [8,75], Gefäß- [28] und Nervenläsionen [8,10,35,75,173], Muskelverletzungen und –herniationen [34], sowie Dislokationen des Sacro-Iliakalgelenkes und Beckenfrakturen [33,52,150] mit Ausbildung von Pseudarthrosen [205] beschrieben. Neben der Entnahmemorbidität weist der autologe trikortikale Beckenkammspan als interkorporelles Implantat auch biologische und mechanische Probleme auf. Die biologische Problematik an der Halswirbelsäule liegt in einer relativ langen Einheilungszeit von ca. 6 Monaten [71,209] und in einer relevanten Pseudarthroserate, die mit 4-11 % der operativ stabilisierten Patienten angegeben wird [71,74,144,209]. Die Pseudarthrosen sind dabei zum Teil auf aseptische Nekrosen des Beckenkammspanimplantats zurückzuführen [174,209]. Besonders die mechanischen Probleme des Beckenkammspanimplantats, wie Implantat-Sinterung, Implantat-Frakturen oder Implantat-Wanderungen mit in der Folge auftretender kyphotischer Deformität der Halswirbelsäule [4,125,174,193,197], führen derzeit zum zunehmenden Ersatz des autologen trikortikalen Beckenkammspans durch Spongiosa augmentierte intervertebrale Cages [200].

1.1.2 Intervertebrale Implantate (Cages)

Seit 1986 verwendet Harms [68] ein metallisches intervertebrales Implantat augmentiert mit autologer Spongiosa zur interkorporellen Spondylodese. 1988 stellte Bagby [6] erstmals einen schraubenförmigen intervertebralen „Cage“ zur lumbalen Wirbelkörperfusion vor. Dabei handelte es sich um einen 30 x 25 mm großen, rostfreien, zylindrischen Stahlkorb (Cage). Seine Studien führte Bagby [6] gemeinsam mit dem Veterinärmediziner Grant [43] an Pferden durch, die an einer Form der spondylarthrotischen Myelopathie, dem Wobbler-Syndrom, litten. Der „Bagby-Korb“ wurde mit autologer Spongiosa gefüllt und nach partieller Diskektomie formschlüssig im Intervertebralraum platziert. Die so operativ versorgten Pferde hatten eine gute Überlebensrate. Einigen war es sogar wieder

möglich Rennen zu gewinnen [43]. Aus diesen Untersuchungen ging letztlich der „Bagby and Kuslich“ (BAK) Cage hervor, der mit primär großem Erfolg klinisch eingesetzt wurde [111]. Seither wurden eine Vielzahl dieser Implantate mit unterschiedlichen Designs bestehend aus verschiedenen Materialien (Stahl, Titan, Karbon, Poly-Ether-Ether-Keton) entwickelt [200]. Diesen Implantaten ist gemeinsam, dass sie, mit der Zielsetzung eine intervertebrale Spondylodese zu erreichen, in das Bandscheibenfach eingebracht werden, wobei durch ihre formschlüssige Verankerung die Notwendigkeit zur additiven Sicherung mittels Spondylodeseplatte entfällt [200]. Zusätzlich sollen intervertebrale Cages die mechanische Stabilität des Bewegungssegmentes und die strukturelle Integrität des Zwischenwirbelraumes während des knöchernen Durchbauungsprozesses gewährleisten [200]. Vor allem durch die biomechanischen Eigenschaften dieser Implantate fanden Cages weite Verbreitung. So konnte gezeigt werden, dass die intraoperativ erzielte Distraktion des Bandscheibenraums durch Cages besser erhalten werden kann als durch autologes Knochenmaterial [165]. Zusätzlich konnte im Vergleich zum Beckenkammspanimplantat in zahlreichen biomechanischen in vitro Untersuchungen an zervikalen und lumbalen Präparaten für verschiedenartige Cagedesigns eine höhere initiale mechanische Stabilität des Bewegungssegmentes nachgewiesen werden [17,21,38,51,72,85,123,136,153,175,176,186,203]. Auch klinische Untersuchungen konnten nachweisen, dass durch Cages die mechanischen Probleme des Beckenkammspanimplantats sowohl an der Hals- als auch Lendenwirbelsäule minimiert werden können [21,66,67,19,111,112,113,152]. Demzufolge wird die intervertebrale Spondylodese mit Spongiosa augmentierten Cages gelegentlich bereits als neuer klinischer „golden standard“ bezeichnet [200]. Trotz der im Vergleich zum autologen trikortikalen Beckenkammspan verbesserten mechanischen Eigenschaften intervertebraler Cages, verbleibt jedoch die biologische Notwendigkeit, die Implantate mit autologer Spongiosa zu augmentieren, um eine sichere intervertebrale Spondylodese zu erzielen [200]. Obwohl die Entnahmemorbidität autologer Beckenkammspongiosa geringer ist als die des trikortikalen Beckenkammspans [35,60,75], kann somit durch Cages allein diese additive Morbidität, die mit der Entnahme des autologen Knochenmaterials verbunden ist, nicht gänzlich vermieden werden.

In klinischen Untersuchungen konnte sowohl an der Hals- als auch an der Lendenwirbelsäule trotz ähnlicher Evaluationsprotokolle eine relevante Variabilität

des operativen Erfolges bei Verwendung verschiedener Cagedesigns demonstriert werden [66,152,155]. Während bei der Verwendung sogenannter Harmscages (Meshed-Titanium-Cages) gute Erfolgsraten beschrieben wurden, zeigten sich bei Verwendung von BAK-Cages hohe Pseudarthrosen- und Revisionsraten [67,152]. Ob dieser Ergebnisvariabilität operative Faktoren oder das Implantatdesign zugrunde liegen, ist derzeit unbekannt.

Nach Weiner [200] lassen sich Cages in 3 Designgruppen einteilen: Schraubendesign (horizontale Zylinder), Boxdesign und Zylinderdesign (vertikale Zylinder). Dabei basieren die derzeit vorliegenden Cagedesigns im Wesentlichen auf empirischen klinischen Erfahrungen und sind wissenschaftlich meist nur ungenügend fundiert. Unter biomechanischen Gesichtspunkten kann die Stabilität des Cage-fixierten Bewegungssegmentes in eine Primärstabilität und eine Sekundärstabilität unterteilt werden. Während die Primärstabilität die direkt postoperativ erzielte Stabilität beschreibt, wird die Sekundärstabilität der Implantate durch deren knöcherne Einheilung erreicht.

Derzeit existieren nur wenige biomechanische in vitro Studien, die die Primärstabilität unterschiedlicher Cagedesigns miteinander vergleichen [140]. Zudem ist die aus diesen Untersuchungen resultierende Datenlage nicht konstant. So konnte Oxland [140] zum Beispiel beim Vergleich von Box- und Zylinderdesign-Cages an der Lendenwirbelsäule keinen Unterschied zwischen diesen Cagedesigns nachweisen. Hingegen zeigte Wilke [203] an der Halswirbelsäule bei ähnlichem Versuchsaufbau eine signifikant höhere mechanische Stabilität von Boxdesign-Cages. Ob verschiedene Cagedesigngruppen einen unterschiedlichen Einfluss auf die biomechanische in vitro Primärstabilität haben, bleibt demzufolge momentan unklar.

Darüber hinaus ist derzeit unbekannt, welcher Zusammenhang zwischen der Primärstabilität eines Cages und der während des knöchernen Einheilungsvorgangs erzielten Sekundärstabilität des Implantats besteht [87]. Vermutet wird, dass eine höhere Primärstabilität durch die „mechanische Ruhe“ im Bewegungssegment zu einem besseren Einheilen des Cages und damit zu einer höheren Sekundärstabilität führt [21,87,102,117,136,153,198].

Die Sekundärstabilität eines Cages ergibt sich einerseits aus der Fähigkeit des Implantats, die strukturelle Integrität des Zwischenwirbelraumes zu erhalten [85,165] und andererseits aus den designspezifischen Charakteristika, den biologischen

Durchbauungsvorgang zu fördern [18,87,200]. Als entscheidender Designparameter für den Erhalt der Zwischenwirbelraumhöhe wurde die Endplattenkonfiguration des Cages definiert [51,79,165]. Hierbei wurde die einfache Gleichung formuliert: Je größer die Auflagefläche des Cages, desto geringer die Sinterung des Cages in vivo [51]. Andererseits weisen experimentelle in vitro Untersuchungen darauf hin, dass die maximale Pore in der Auflagefläche eines Cages eine entscheidende Funktion für den knöchernen Durchbauungsvorgang aufweist [25,29,87]. Kanayama [87] konnte in in vitro Untersuchungen nachweisen, dass eine größere kontinuierliche Pore in der Auflagefläche zu einer Reduktion der Stress-Protektion der inkorporierten Spongiosa im Cage führt. Des weiteren vermutete er, dass es durch die Reduktion der Stress-Protektion zu einer mechanischen Stimulation der Spongiosa innerhalb des Cages kommt und somit das Einheilungsverhalten des Cages in vivo verbessert würde [87].

Die oben genannten Anforderungsprofile an das Design eines Cages stehen sich konkurrierend gegenüber. Ein ideales Cagedesign müsste demzufolge sowohl eine maximale Auflagefläche aufweisen, um eine Sinterung des Implantates zu vermeiden, als auch eine maximale Pore in der Auflagefläche besitzen, um das Einheilungsverhalten des Cages zu optimieren. In vivo Untersuchungen, die das Einheilungsverhalten eines Cages in Relation zu oben genannten Designparametern evaluieren, existieren derzeit nicht.

1.2 Wachstumsfaktoren

Vor mehr als 30 Jahren konnte Urist [192] erstmals die osteoinduktiven Eigenschaften demineralisierter Knochenmatrix nachweisen. Technische Fortschritte in der Protein-Isolation und der molekularen Klonierung führten zur Entdeckung zahlreicher Wachstumsfaktoren. Diese löslichen niedermolekularen Proteine können in folgende Familien unterteilt werden [118,191]: transforming growth factors (TGFs) inklusive bone morphogenetic proteins (BMPs), platelet derived growth factors (PDGFs), insulin like growth factors (IGFs), fibroblast growth factors (FGFs) und epidermal growth factors (EGFs). Unter Zuhilfenahme gentechnisch modifizierter Zelllinien werden mittlerweile eine Vielzahl dieser Wachstumsfaktoren in rekombinanter humaner (rh) Form hergestellt. Jedoch konnte bisher nur für wenige dieser Wachstumsfaktoren ein signifikanter osteoinduktiver Effekt nachgewiesen werden [118,191].

In der Wirbelsäulenchirurgie liegt die klinische Zielsetzung der Wachstumsfaktorenapplikation in der Stimulation der intervertebralen Spondylodese [12,163]. Dadurch soll die Fusion beschleunigt und die Pseudarthroserate gesenkt werden. Zusätzlich soll durch die Wachstumsfaktorenapplikation die Notwendigkeit zur Entnahme autologen Knochenmaterials und die damit verbundene Entnahmemorbidität vermieden werden [42,53,61]. Durch das so optimierte Therapieverfahren sollen die Rehabilitation der Patienten forciert, die Komplikationsrate gesenkt und somit die Kosten der Behandlung reduziert werden [13].

1.2.1 BMP-2 und BMP-7

In der experimentellen Wirbelsäulenchirurgie konnten trotz zahlreicher Forschungsbemühungen bisher nur die Wachstumsfaktoren BMP-2 [11,12,13,42,53,70,84,105,127,131,164,162,166,182,208,210] und BMP-7 (OP-1) [36,39,61,115,126,142,143,145] einen signifikanten osteoinduktiven Effekt unter Beweis stellen. BMP-2 und BMP-7 sind Mitglieder der TGF-β Familie und gehören, in der derzeit 15 Mitglieder umfassenden BMP-Familie, zu den am besten charakterisierten BMPs [24,46]. Beide Wachstumsfaktoren sind im Tierreich weit verbreitet und erfüllen zahlreiche Funktionen bei der Proliferation und Differenzierung unterschiedlichster Gewebe [24,46]. So wurden Effekte auf die frühe Phase der embryonalen Skelettentwicklung, die Morphogenese und Organogenese beschrieben [24,46]. Entdeckt wurden beide Wachstumsfaktoren jedoch aufgrund ihrer Fähigkeit zur Knocheninduktion und Knochenregeneration [192]. BMP-2 und BMP-7 sind beispielsweise in der Lage eine de novo Synthese von Knochen in ektopischen Weichteilgewebe sogar in Abwesenheit von Knochenmarkselementen hervorzurufen [5,199]. Folglich konnte im Vergleich zu autologem Knochenmaterial für beide Wachstumsfaktoren in zahlreichen tierexperimentellen Untersuchungen eine beschleunigte und effektivere Knochenheilung nachgewiesen werden [105,127,131,164,162,166,182,208,210,36,39,61,115,126,142,143]. In der experimentellen Wirbelsäulenfusion stellt derzeit der Wachstumsfaktor BMP-2 aufgrund seiner hohen osteoinduktiven Kapazität den „golden standard“ dar und wird bereits kombiniert mit einem Kollagen Trägersystem in ersten klinischen Studien untersucht [13,14]. Obwohl diese spezifischen osteoinduktiven Eigenschaften von

BMP-2 gelegentlich für unabdingbar gehalten werden [42,53,70], um eine solide knöcherne Spondylodese zu erzielen, können diese Charakteristika speziell bei spinaler Applikation auch unerwünschte Folgen haben. So konnte gezeigt werden, dass BMP-2 induzierte Ossifikationen im Ligamentum flavum zu einer Verdrängung des Myelons führten [133]. Hoshi [81] wies darüber hinaus nach, dass BMP-2 induzierte Ossifikation spinaler Ligamente, speziell des Lig. longitudinale posterior, zu einer neurologisch wirksamen Myelonkompression führen können. Andere Autoren konnten zeigen, dass bei der gehäuft in Ostasien auftretenden hereditären OPLL (ossification of the posterior longitudinal ligament), die mit einer Myelopathie einhergeht, BMP-2 pathophysiologisch eine entscheidende Bedeutung zukommt [81,106]. Aufgrund der Vielzahl existierender Wachstumsfaktoren und Kombinationsmöglichkeiten muss derzeit offen bleiben, ob BMP-2 oder BMP-7 die „optimalen" Wachstumsfaktoren zur Stimulation der intervertebralen Spondylodese sind, besonders wenn berücksichtigt wird, dass derzeit keine vergleichenden Untersuchungen zur Wirksamkeit verschiedener Wachstumsfaktoren bei der intervertebralen Spondylodese vorliegen.

1.2.2 IGF-I und TGF-β1

Kürzlich konnten in vitro und in vivo Untersuchungen einen signifikant osteoinduktiven Effekt sowohl für die isolierte als auch für die kombinierte Applikation von IGF-I und TGF-β1 nachweisen [83,118,119,134,154,171, 172,188,191].

Die Insuline-like growth factors (IGFs), auch Somatomedine oder Scelatal growth factors genannt, erfüllen wesentliche Funktionen innerhalb des Knochenstoffwechsels. Bislang wurden zwei IGFs charakterisiert: IGF-I (Somatomedin-C) und IGF–II (Scelatal growth factor) [134,156]. Beide IGFs verfügen über die gleichen biologischen Wirkungen, jedoch ist IGF-I vier bis sieben mal potenter als IGF-II [78]. In zahlreichen Untersuchungen konnte nachgewiesen werden, dass IGF-I in vitro die Angiogenese, die Proliferation und Differenzierung von Osteoprogenitorzellen, die Proliferation und Replikation von Osteoblasten und schließlich die Formation der Knochenmatrix stimuliert [78,170,194,196].

TGF-β1 gehört gemeinsam mit den BMPs zur TGF-β Familie [26]. Bislang sind fünf verschiedene Subtypen dieser Familie (TGF-β1-5) bekannt [26]. TGF-β1 reguliert

eine Vielzahl unterschiedlicher Zellen, wie zum Beispiel mesenchymale Vorläuferzellen, Osteoblasten, Osteoklasten und Chondrozyten, die direkt oder indirekt an der Ossifikation und am Remodeling des Knochens beteiligt sind [147,157]. Von besonderer Bedeutung ist dabei die stimulierende Wirkung von TGF-β1 auf die Differenzierung von Osteoprogenitorzellen zu Osteoblasten [30] und auf die Proliferation und Teilung von Osteoblasten und osteoblastenähnlichen Zellen [86,160]. IGF-I und TGF-β1 haben viele gemeinsame, teilweise synergistische Effekte auf den Knochenstoffwechsel. In vivo Untersuchungen konnten beispielhaft nachweisen, dass erniedrigte Serumspiegel von IGF-I oder TGF-β1 mit Knochenverlust und Osteoporose assoziiert sind [2,57,206], wohingegen die isolierte Applikation von IGF-I und TGF-β1 zu einer Stimulation der Frakturheilung führt [83,137,181]. Die in vivo nachweisbaren Serum-Konzentrationen von IGF-I und TGF-β1 zeigen sowohl gemeinsame altersabhängige Schwankungen als auch gleichsinnige Veränderungen während des Knochenwachstums oder nach Stimulation durch PTH (Parathormon) [76,148,149]. Zusätzlich scheint eine Interaktion zwischen den beiden Wachstumsfaktoren zu existieren. TGF-β1 stimuliert beispielsweise Osteoblasten zur Bildung von IGF-I [122], während in Abhängigkeit von der Zellart sowohl stimulierende als auch hemmende Effekte von TGF-β1 auf die IGF-I Synthese beschrieben wurden [49,190,120]. Des weiteren konnte für die kombinierte Applikation von IGF-I und TGF-β1 ein größerer stimulierender Effekt auf die Ausbildung der Knochenmatrixformation nachgewiesen werden [124,172] als für die jeweilige Einzelapplikation [118,147].

Von besonderer Bedeutung bei spinaler Applikation von IGF-I und TGF-β1 ist, dass im Gegensatz zu BMP-2 beide Wachstumsfaktoren nicht zur de novo Synthese von Knochen in der Lage sind [118]. Vielmehr liegt die Wirksamkeit dieser Wachstumsfaktoren in der Förderung und Beschleunigung des natürlichen Knochenneubildungspotentials [57]. Da das natürlichen Knochen-neubildungspotentials anatomischen Grenzen respektiert, erscheint eine ektopische Ausbildung von Knochen, zum Beispiel in spinalen Ligamenten mit daraus resultierenden neurologischen Störungen, aufgrund dieses Wirkungsmechanismus der Wachstumsfaktoren unwahrscheinlich.

Experimentelle Untersuchungen zur kombinierten Applikation von IGF-I und TGF-β1 bei der intervertebralen Spondylodese liegen derzeit nicht vor.

1.3 Trägermaterial (Carrier-Systeme)

Ein ideales Carrier-System zur intervertebralen Applikation von Wachstumsfaktoren sollte kontrolliert applizierbar sein, eine kontinuierliche, definierte und lokale Applikation von Wachstumsfaktoren gewährleisten und dabei ohne Nebenwirkungen vollständig resorbierbar sein [14,16,58,59,162]. Die optimale Methode zur lokalen Applikation von Wachstumsfaktoren in der Wirbelsäulenchirurgie wird aufgrund dieses Anforderungsprofils immer noch kontrovers diskutiert.

1.3.1 Kollagen-Carrier

Derzeit werden vorwiegend Kollagenschwämme zur Applikation von Wachstumsfaktoren in experimenteller und klinischer Forschung eingesetzt [12,13,14,53,70,84,127,131,208,210]. Speziell die Kombination eines Kollagen-Carriers mit BMP-2 wird in der Wirbelsäulenchirurgie bereits klinisch erprobt [13]. Jedoch ist die Sicherheit und die Freisetzungskinetik des Kollagen-Carriers fragwürdig [127,167,180,184]. Eine schnelle und unkontrollierte Freisetzung von Wachstumsfaktoren aus Kollagenschwämmen mit unerwünschten Reaktionen des umgebenden Gewebes wurde beschrieben [180]. Auch die intraoperative Platzierung des mit Wachstumsfaktoren benetzten Carriers ist häufig nicht mit der gewünschten Genauigkeit zu erzielen und kann in der Folge zu Ineffektivität und unerwünschten Wirkungen des Wachstumsfaktors führen [5,127]. Martin [127] konnte darüber hinaus in einem Tiermodell zur intertransversalen Fusion der Lendenwirbelsäule zeigen, dass durch die Kompression des Carriers in der paravertebralen Muskulatur der Effekt des Wachstumsfaktors ausblieb. Boden [12] wies darauf hin, dass der Kollagenschwamm zwar für intertransversale Fusionsmodelle in Kaninchen und Hunden geeignet war, aber im Affen-Modell versagte. Schließlich ist der Sicherheitsaspekt des aus bovinen Material hergestellten Kollagenschwamms hinsichtlich allergischer Reaktionen und nicht zuletzt der Übertragung von Infektionskrankheiten fraglich [184].

1.3.2 Polylaktid-Carrier

Als Alternative stehen derzeit biodegradierbare Trägersysteme wie Poly-Laktidsäuren (PLA), Poly-Glykolidsäuren (PGA) und deren Kopolymere zur

Verfügung [42,53,114,161,163]. In vorangegangenen Untersuchungen, besonders bei Verwendung von Trägersysteme aus PLA und PGA, konnte jedoch gezeigt werden [53,107,109,132], dass Degradationsprodukte der Polymere zur Alteration der biologischen Umgebungsbedingungen führen können [114,128]. Speziell inflammatorische Reaktionen mit begleitender Ausbildung von Osteolysen wurden beschrieben [53]. Diese Fremdkörperreaktionen sind dabei aber entscheidend von der Menge und der Art des biodegradierbaren Trägermaterials (reines Polymer oder Kopolymer) sowie von den lokalen Milieubedingungen (Durchblutung, Inflammation) abhängig. Dies erklärt die uneinheitliche Studienlage bei vergleichenden Untersuchungen zwischen Kollagen und PLA bzw. PGA Carrier-Systemen. David [42] fand beim Vergleich von PLA und Kollagen Carrier eine effektivere lumbale Spondylodese mit dem Kollagen Carrier. Fischgrund [53] konnte in einem intertransversalen Wirbelsäulenfusionsmodell keinen Unterschied zwischen der Effektivität eines Kollagenschwamm- und PGA- Carriers nachweisen. Jedoch erzielten Zegzula [210] und Ozuna [141] bei Verwendung eines Poly-(D,L-laktid-coglykolide) (PLA/PGA)-Carriers in einem intertransversalen Wirbelsäulenfusionsmodell bessere Ergebnisse als bei Verwendung eines Kollagen-Carriers. Kürzlich wurde eine kalte Beschichtungstechnologie entwickelt, die es erlaubt, Implantate mit einer 0,01 mm dicken Schicht aus Trägermaterial zu überziehen [73,171]. In diese biodegradierbare Poly-(D,L-laktid) (PDLLA)-Beschichtung können biologisch aktive Substanzen (z. B. Wachstumsfaktoren) integriert werden, die während der Degradation des Biomaterials kontinuierlich und lokal freigesetzt werden [171]. Speziell die dadurch ermöglichte Kombination von etablierten Wirbelsäulenimplantaten mit biologisch wirksamen Substanzen und die geringe Beschichtungsmenge, sowie die hohe mechanische Stabilität dieses Trägermaterials [171] machen die Verwendung dieser Technologie in der Wirbelsäulenchirurgie attraktiv. Ergebnisse zur Verwendung dieses Carrier Systems in der Wirbelsäulenchirurgie liegen derzeit aber noch nicht vor.

1.4 Tiermodell

Frische humane Präparate für in vitro Experimente sind nur schwer zugänglich. Tierpräparate sind einfacher verfügbar und zeigen eine bessere Vergleichbarkeit wenn sie nach Rasse, Geschlecht, Alter und Gewicht ausgewählt werden

[27,201,202]. Demzufolge wurden zunehmend mehr in vitro und in vivo Tiermodelle für die humane Wirbelsäule etabliert [1,11,12,36,39,53,61,65,70,84,127, 130,131,135,139,178,208,210]. Obwohl die humane Lendenwirbelsäule den Wirbelsäulenbereich darstellt, der am häufigsten einer operativen Therapie bedarf, sind Tiermodelle an der Lendenwirbelsäule aus zahlreichen Gründen umstritten [179]. Während die Wirbelsäule von Vierfüßlern kyphotisch ist, ist die humane Lendenwirbelsäule lordotisch. Dieser Unterschied im sagitalen Wirbelsäulenprofil der Spezies führt auch zu deutlichen biomechanischen Unterschieden in der nativen Beweglichkeit der Lendenwirbelsäulen und im Rahmen der biomechanischen Beanspruchung auch zur Ausbildung unterschiedlicher Knochentextur und –dichte [179]. Schließlich sind die Unterschiede in der lumbalen Bandscheibenraumhöhe evident und erlauben daher keine Evaluation original großer humaner Lendenwirbelsäulenimplantaten in vielen lumbalen Tiermodellen [201,202]. Folglich fanden zur Klärung experimenteller Fragestellungen zunehmend zervikale Tiermodelle Verbreitung. Neben Ziegen, Schweinen, Hunden und Kälbern, wurden vor allem Schafe als Versuchstiere für die Wirbelsäulenforschung herangezogen [1,65,116,130,135,139,178,207]. Von entscheidender Bedeutung für die Aussagekraft einer tierexperimentellen Evaluation der Schafshalswirbelsäule ist aber eine genaue Kenntnis der Relation zwischen Schafs- und humaner Wirbelsäule. Obwohl zahlreiche Untersuchungen zur Anatomie und Mechanik der humanen Halswirbelsäule vorliegen [27,40,47,48,56,101,129], existieren bisher nur zwei Untersuchungen der Schafshalswirbelsäule. Wilke [201,202] führte Untersuchungen von Schafs-Präparaten durch und verglich die Ergebnisse mit Literaturdaten der humanen Halswirbelsäule. Dabei konnte er zwar große anatomische Unterschiede, aber eine gute Vergleichbarkeit zwischen den beiden Spezies hinsichtlich biomechanischer Parameter wie Bewegungsumfang, Steifigkeit und neutraler und elastischer Zone nachweisen [201,202]. Direkt vergleichende Untersuchungen zwischen humaner und Schafshalswirbelsäule zur funktionellen Anatomie, Funktions-Röntgenanatomie und Knochendichte mit gleichen Evaluationsparametern fehlen in der Literatur vollständig. Besonders das für experimentelle Untersuchungen heranzuziehende zervikale Bewegungssegment der Schafshalswirbelsäule ist umstritten.

1.5 Wissenschaftliche Fragestellung

Die Ablösung der klassischen intervertebralen Spondylodese mit trikortikalem Beckenkammspan durch intervertebrale Implantate, sogenannte Cages, wirft Fragen zum Zusammenhang zwischen dem Design der Implantate und deren Auswirkung auf die intervertebrale Fusion auf. Obwohl mechanische Probleme des Beckenkammspanimplantates durch Cages bereits jetzt reduziert werden konnten, verbleibt derzeit die Notwendigkeit die Implantate mit autologem Knochenmaterial zu füllen, um eine sichere Spondylodese zu erzielen. Die mit der Entnahme des autologen Knochenmaterials assoziierte Entnahmemorbidität stellt ein zentrales Problem der Wirbelsäulenchirurgie dar. Erste Versuche durch die Applikation von Wachstumsfaktoren wie BMP-2 und BMP-7 mittels eines Kollagen-Carriers diese Entnahmemorbidität zu eliminieren waren zwar im Bezug auf die Fusionsresultate erfolgreich, brachten jedoch die oben angegebenen Probleme mit sich. Kenntnisse über Designparameter, die eine intervertebrale Spondylodese determinieren, sowie alternative Wachstumsfaktoren und Carrier-Systeme könnten zu einer Verbesserung der Ergebnisse der zervikalen intervertebralen Spondylodese beitragen.

Zu Beginn der Planungsphase (1998) der nachfolgend geschilderten experimentellen Untersuchungen und mit zunehmendem Informationsgewinn waren daher die folgenden Fragen offen bzw. haben sich im Verlauf entwickelt:

1. Eignet sich die Schafshalswirbelsäule als Modell der humanen Halswirbelsäule?
2. Bestehen biomechanische Unterschiede in der in vitro Primärstabilität verschiedener Cagedesigns?
3. Bestehen Unterschiede im Einheilungsverhalten verschiedener Cagedesigns?
4. Welche Designparameter haben Einfluss auf die in vivo Sekundärstabilität verschiedener Cagedesigns?
5. Wie verhält sich der Poly(D,L-laktid) Carrier im Vergleich zum Kollagen-Carrier?
6. Ist die kombinierte Applikation von IGF-I und TGF-β1 mittels Poly(D,L-laktid) beschichtetem Cage in der Lage die intervertebrale Spondylodese zu stimulieren?
7. Welcher Dosis-Wirkungs-Zusammenhang besteht bei der kombinierten

Applikation von IGF-I und TGF-β1 mittels Poly(D,L-laktid) beschichtetem Cage?

8. Ist die intervertebrale Spondylodese durch kombinierte Applikation von IGF-I und TGF-β1 mittels Poly(D,L-laktid) beschichtetem Cage ebenso effektiv wie die Spondylodese mit autologem Knochenmaterial?
9. Wie verhält sich die kombinierte Applikation von IGF-I und TGF-β1 im Vergleich zur BMP-2 Applikation?

Entsprechend diesen Fragestellungen wurden die Effekte des Cagedesigns, der Wachstumsfaktoren und der Carrier-Systeme auf die intervertebrale Spondylodese in einer anatomisch-biomechanischen, einer rein biomechanischen und sechs tierexperimentellen Teilstudien untersucht.

2 Experimentelle Untersuchungen

2.1 Tiermodell Schafshalswirbelsäule

Für die Durchführung und Interpretation tierexperimenteller Untersuchungen ist die Wahl des adäquaten Tiermodels entscheidend. Obwohl die Schafshalswirbelsäule als Tiermodell der humanen Halswirbelsäule bereits vor dieser Untersuchung etabliert war [201], ist die bestehende Datenlage nicht dazu geeignet, eine zuverlässige Interpretation der im Tierexperiment gewonnenen Ergebnisse zu gewährleisten. Im Einzelnen wurden daher folgende Fragestellungen in dieser anatomisch-biomechanischen Untersuchung adressiert.

- Für die folgenden tierexperimentellen Untersuchungen waren humane Implantate vorgesehen. Daher war es notwendig zu klären, ob die Höhe und Breite des Bandscheibenraums sowie der angrenzenden Deck- und Bodenplatten der Wirbelkörper der Schafshalswirbelsäule dazu geeignet sind, human große zervikale Implantate aufzunehmen.
- Da eine biomechanische in vitro Evaluation zervikaler Cages geplant wurde, war es notwendig, die Bewegungssegmente der Schafshalswirbelsäule hinsichtlich ihrer biomechanischen Kenngrößen Bewegungsumfang, Steifigkeit, neutrale und elastische Zone mit den humanen Bewegungssegmenten zu vergleichen.
- Die Knochendichten der Deck- und Bodenplatten der Wirbelkörper sind für das in vivo Sinterungsverhalten von intervertebralen Cages von großer Bedeutung [69,85,103,123]. Daher war es notwendig zu klären, welche Unterschiede zwischen humaner und Schafs-Halswirbelsäule in der Knochendichte der Wirbelkörper bestehen.
- Funktionsradiologische Parameter in maximaler Flexion und Extension erlauben beim Menschen die Unterscheidung zwischen einem fusionierten und einem nicht fusioniertem Bewegungssegment [111,138]. Demzufolge war es erforderlich, Unterschiede in den segmentalen funktionsradiologischen Parametern zwischen den Spezies zu erheben.
- Schließlich war es das Ziel dieser Untersuchung, bei entsprechender Eignung des Tiermodells, in der Zusammenfassung aller Ergebnisse das Bewegungssegment auszuwählen, dass eine möglichst gute Übereinstimmung zwischen den Spezies zeigt.

SPINE Volume 26, Number 9, pp 1028–1037

Comparison Between Sheep and Human Cervical Spines

An Anatomic, Radiographic, Bone Mineral Density, and Biomechanical Study

Frank Kandziora, MD,* Robert Pflugmacher,* Matti Scholz,* Klaus Schnake, MD,* Martin Lucke, MD,* Ralf Schröder, MD,† and Thomas Mittlmeier, MD*

Study Design. The quantitative anatomic, radiographic, computerized tomographic, and biomechanical data of sheep and human cervical spines were evaluated.

Objectives. To compare the anatomic, radiographic, computerized tomographic, and biomechanical data of human and sheep cervical spines to determine whether the sheep spine is a suitable model for human spine research.

Summary of Background Data. Sheep spines have been used in several *in vivo* and *in vitro* experiments. Quantitative data of the normal sheep cervical spine are lacking, yet these data are crucial to discussion about the results of such animal studies.

Methods. In this study, 20 fresh adult female Merino sheep cervical spines and 20 fresh human cadaver cervical spines were evaluated anatomically, radiographically, computerized tomographically, and biomechanically. Three linear and two angular parameters were evaluated on four digital radiographic views: anteroposterior, right lateral in neutral position, flexion, and extension. Quantitative computed tomography scans at the center of each vertebral body and 3 mm below both endplates were analyzed for bone mineral density measurements. Biomechanical testing was performed in flexion, extension, axial rotation, and lateral bending by a nondestructive stiffness method using a nonconstrained testing apparatus. Range of motion and stiffness of each motion segment were calculated. Additionally, 10 linear anatomic parameters of each vertebra were measured using a digital ruler.

Results. Anterior and mean disc space height in the sheep cervical spine increased constantly from C2–C3 to C6–C7, whereas middle disc space height decreased and posterior disc space height remained unchanged. Anterior and mean disc space height were significantly higher in sheep. In both sheep and human cervical spines, intervertebral angles were not significantly different. Standard deviations of bone mineral density in the human cervical spine were fourfold higher than in the sheep cervical spine, yet no significant differences were found in bone mineral density values between the two species. Range of motion differed significantly between the two species except in flexion–extension of C3–C4, C5–C6, axial rotation of C2–C3, and lateral bending of C2–C3, C3–C4, and C4–C5. Stiffness also was significantly different except in flexion–extension of C2–C3, C4–C5, C5–C6, and lateral bending of C2–C3, C3–C4, and C4–C5. Anatomic evaluation showed no difference in upper endplate parameters for C4 and C5.

Conclusions. Although several differences were found between human and sheep cervical spines, the small intergroup standard deviations and the good comparability with the human spine encourage the use of the sheep cervical spine as a model for cervical spine research. On the basis of the quantitative data obtained in this study, the sheep motion segment C3–C4 seemed to be the most reliable model for the corresponding human motion segment. [Key words: anatomy, animal model, biomechanics, bone mineral density, cervical spine, human, radiology, sheep] **Spine 2001;26:1028–1037**

Fresh human cadaveric specimens are difficult to obtain for *in vitro* experiments. Animal specimens are more easily available and show much better homogeneity than human specimens when selected for breed, gender, age, and weight.[7,8,12] Therefore, animals are becoming more and more common as *in vivo* and *in vitro* models for the human spine. Besides goat, pig, dog, and calf spines, most studies in the past have been carried out with sheep spines. The sheep cervical spine is stated to be a good model for the human cervical spine, mainly because of the comparable cervical lordosis. Therefore, sheep spines have been used in several *in vivo* and *in vitro* experiments[1,3,11,16,18,20,21,24,25,30,34–36].

Quantitative data of the normal sheep cervical spine are lacking, but they are crucial for discussion about the results of such animal studies. Therefore, this study aimed to determine quantitative anatomic, radiographic, computerized tomographic (CT), and biomechanical data of the sheep cervical spine and compare it directly with the corresponding data of the human spine. The database generated in this study may be helpful for planning experimental studies using a sheep cervical spine model.

Materials and Methods

In this study, 20 fresh adult (age, 2 years; average weight, 64.6 ± 3.7 kg) female Merino sheep cervical spines (C0–TH1) and 20 fresh human cadaver cervical spines (C0–TH1) were evaluated anatomically, radiographically, computerized tomographically, and biomechanically. The average age of the human donors (10 women and 10 men) was 51.2 ± 5.9 years (range, 32–60 years). The medical history of each human do-

From the *Unfall und Wiederherstellungschirurgie and the †Strahlenklinik and Poliklinik, Universitätsklinikum Charité der Humboldt Universität Berlin, Campus Virchow-Klinikum, Augustenburgeplatz 1, 13353 Berlin, Germany.
Acknowledgment date: April 4, 2000.
Acceptance date: July 28, 2000.
Device status category: 1.
Conflict of interest category: 12.

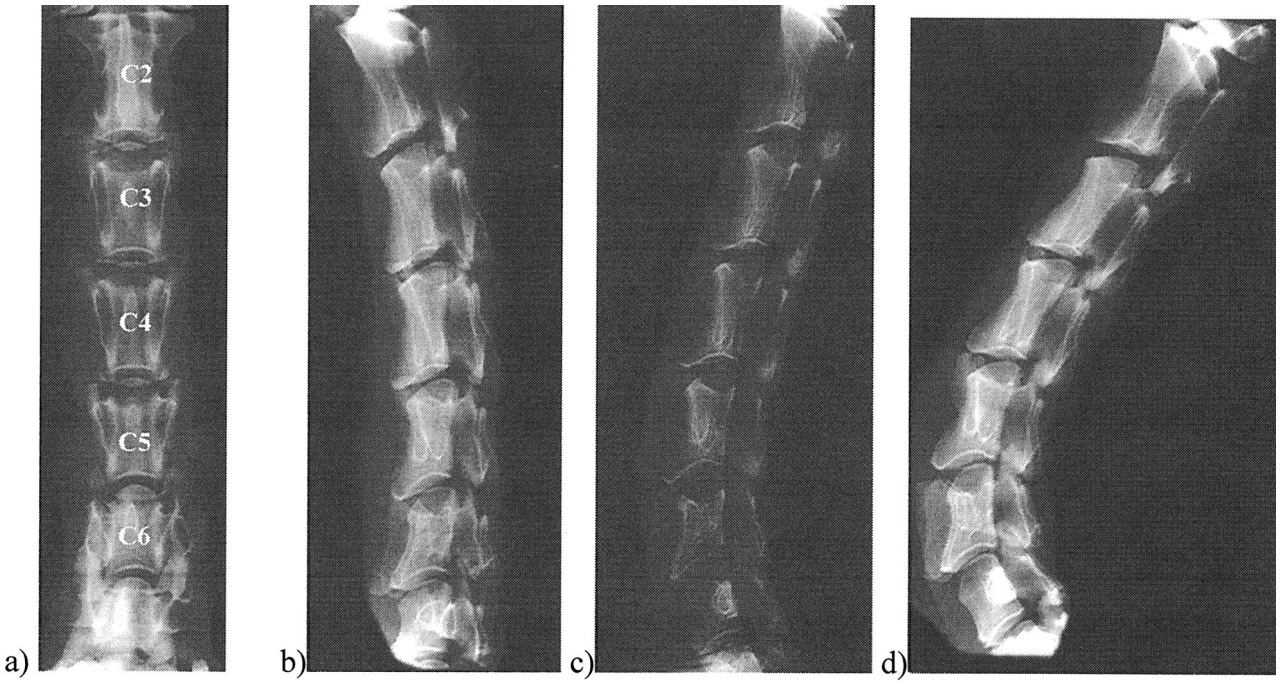


Figure 1. Digital radiographic scans of the sheep cervical spine in four projections: **a,** anteroposterior, **b,** right lateral in flexion, **c,** right lateral in neutral position, and **d,** right lateral in extension.

nor was reviewed to exclude trauma malignancy or metabolic disease that might compromise functional or anatomic parameters of the cervical spine.

Radiologic Measurements of Sheep and Human Cervical Spines. Digital radiographic views (100-cm focus-film-distance; 47 kV; 4 mAs) of all the sheep and human cervical spines were performed in four projections: anteroposterior, right lateral in neutral position, flexion, and extension (Figure 1). Three linear and two angular parameters (Figure 2) were measured for every motion segment on each radiographic scan using a digital ruler (accuracy 0.1 mm, inha GmbH, Berlin, Germany) and a goniometer (accuracy 0.5°, inha GmbH). To allow functional radiologic evaluation, TH1 was fixed with pins rigidly while a 60-Nm load was applied to C1 to induce flexion or extension. The magnification of radiographic films as compared with anatomic measurements was taken into consideration. Each value obtained by radiographic measurement was

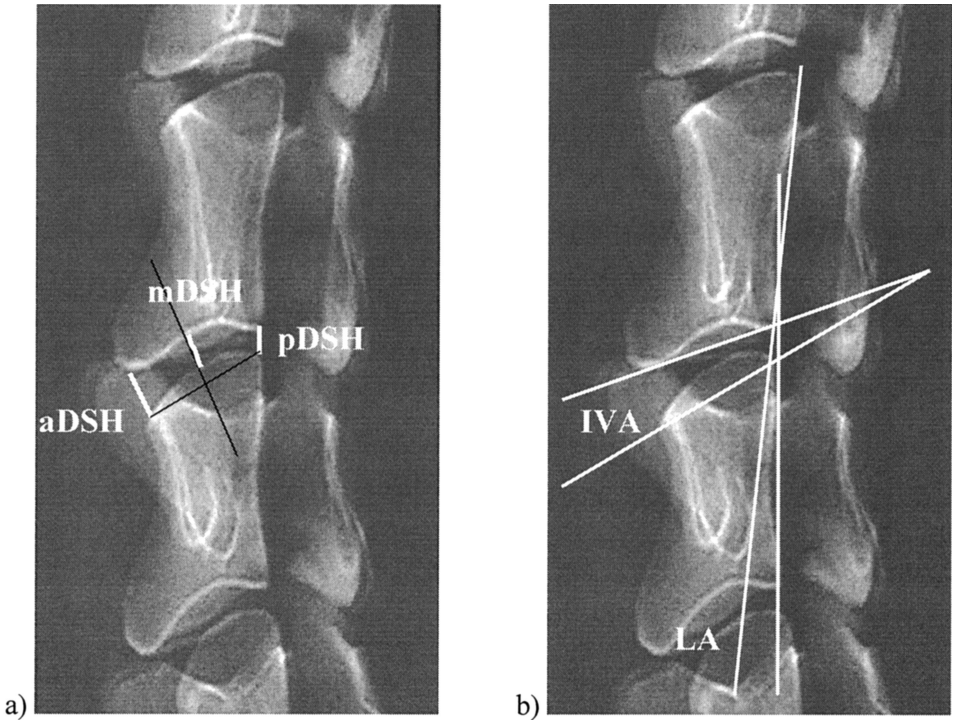


Figure 2. Radiographic measurements. **a,** Anterior disc space height (aDSH), posterior disc space height (pDSH), and middle disc space height (mDSH); **b,** intervertebral angle (IVA) and lordosis angle (LA).

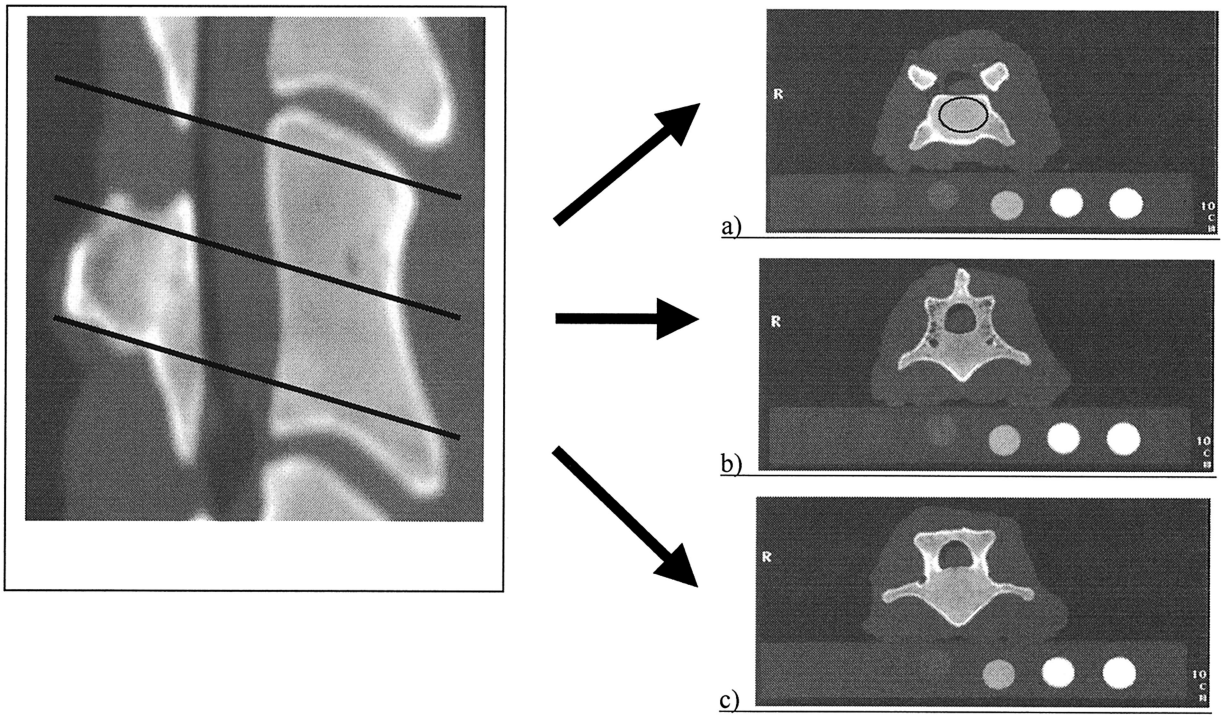


Figure 3. Bone mineral density measurements of the sheep cervical spine. Quantitative computed tomography (CT) scans were obtained in 1-mm cuts. Midsagittal two-dimensional reconstruction of C4 is depicted on the left side. Evaluation was performed on three slices: **a,** upper endplate (3 mm below the cortex), **b,** center of the vertebral body, and **c,** lower endplate (3 mm above the cortex). A 6-point bone mineral density phantom also is shown. The region of interest (ROI), depicted in **a,** was similar for each slice.

multiplied with a correction factor: correction factor = (focus-film-distance − focus-object-distance)/focus-film-distance.

Bone Mineral Density Measurements of Sheep and Human Cervical Spines. Quantitative CT scans for bone mineral density measurements were performed using a Siemens Somatom plus 4 scanner (Siemens Inc., Erlangen, Germany). Slices 1 mm thick were cut parallel to a plane defined by the most anteroinferior point of the endplate in the midsagittal plane and the most inferior point of the right and left inferior facet joint surfaces of C3–C7. Bone mineral density (BMD) measurements were calibrated with a 6-point bone mineral density phantom. In each vertebra (C3–C7), analogous regions of interest were measured at the center of the vertebral body and 3 mm below both endplates (Figure 3). Measurements were performed using specific scanner software (Sienet Magic View VA 30A, Siemens, Inc., Erlangen, Germany).

Stiffness Tests of Sheep and Human Cervical Spines. *En bloc* specimens were stored at −20 C until they were thawed in a water bath at 25 C for biomechanical testing. The motion segments C2 to TH1 were isolated, and superficial musculature was removed. Care was taken to preserve all ligaments.

Each motion segment (C2–C3, C3–C4, C4–C5, C5–C6, C6–C7) was tested separately. Specimens were kept moist during tests. Testing was performed by a nondestructive stiffness method using the nonconstrained testing apparatus (Figure 4) described in detail earlier.[4,13] Pure bending moments were applied using a system of cables and pulleys to induce flexion, extension, left and right lateral bending, and left and right axial rotation. Tension was applied to the cables with a uniaxial testing machine (Zwick 1456, Zwick GmbH, Ulm, Germany). Applied forces were measured with an axial load cell (Z12, HBM, Darmstadt, Germany) mounted on the testing frame. Moments were calculated by multiplying the applied force by the radius of the pulley on the spine-testing fixture.

Three-dimensional displacement of each motion segment was measured using an optical measurement system (Qualysis Inc., Sävebalden, Sweden). Nonlinear diodes (Qualysis) were attached to the corpora of each vertebra. Marker positions were detected with two cameras and recorded with a computerized motion analysis system (PC-Reflex; Qualysis). Angular displacement of upper vertebra in relation to the lower vertebra was calculated from the marker position using custom-made computer software. The average experimental error associated with this method was ±0.17° for flexion–extension, ±0.21° for axial rotation, and ±0.18° for lateral bending [15].

The vertebrae were mounted in pots using polymethylmethacrylate (Technovit 3040; Heraeus Kulzer GmbH, Wehrheim/Ts, Germany). The lower pot was attached rigidly to the base of the testing apparatus. This test setup resulted in a compressive preload of 25 N, attributable to the weight of the upper fixation pot, which represented the average weight of the sheep's head. Moments were applied quasi-statically in increments of 1 Nm to a maximum of 6 Nm. Test modes were flexion, extension, left and right axial rotation, and left and right lateral bending. Specimens were preconditioned using three cycles of a 6-Nm load with a velocity of 1.2 mm/second of the traverse bar of the uniaxial testing machine. The fourth cycle was measured. The total range of motion was measured in

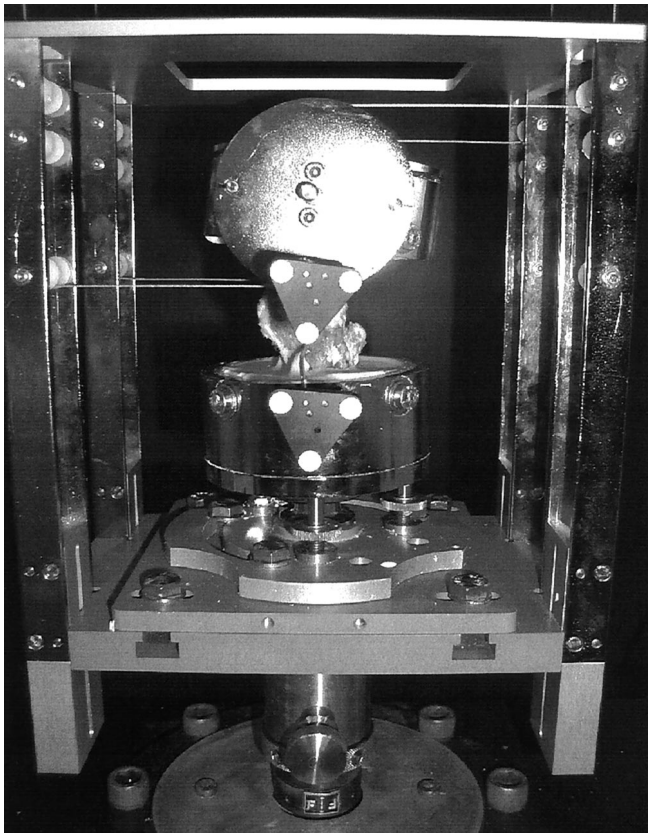


Figure 4. Biomechanical test setup showing the nonconstrained testing apparatus described in detail earlier.[5,13] Pure bending moments were applied using a system of cables and pulleys. The three-dimensional displacement of each motion segment was measured using an optical measurement system (Qualysis Inc., Sävebalden, Sweden). Nonlinear diodes (Qualysis) were attached to the corpora of each vertebra.

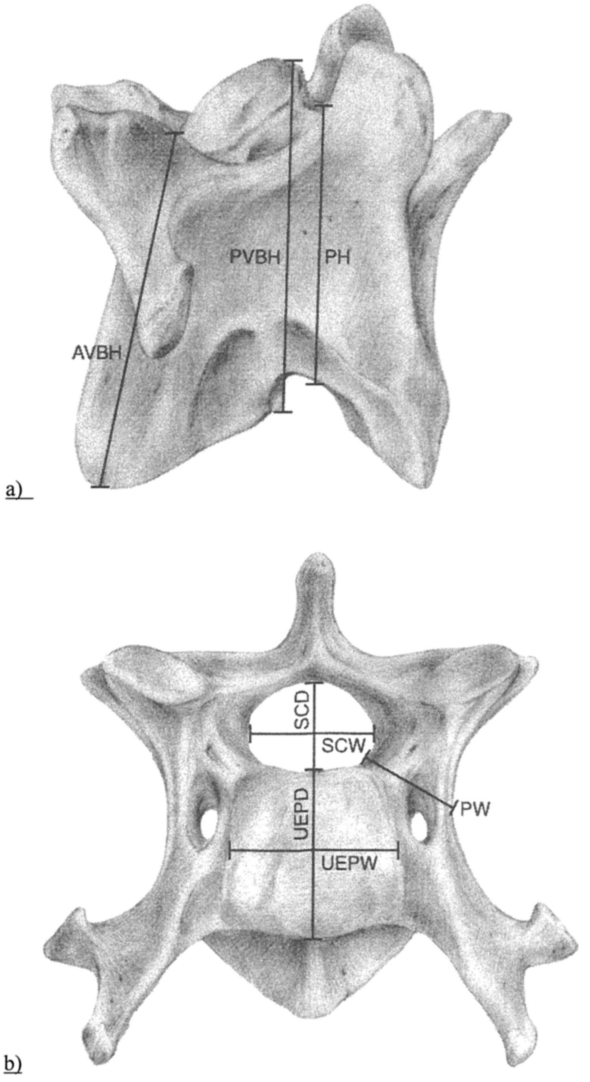


Figure 5. Anatomic measurements. **a,** Anterior vertebral body height (AVBH), posterior vertebral body height (PVBH), and pedicle height (PH). **b,** Upper endplate depth (UEPD), upper endplate width (UEPW), spinal canal depth (SCD), spinal canal width (SCW), and pedicle width (PW). Not shown: lower endplate depth (LEPD) and lower endplate width (LEPW).

degrees. The mean apparent stiffness values in the elastic zone were calculated from the corresponding load-displacement curves.

Anatomic Measurements of Sheep and Human Cervical Spines. Finally, the complete soft tissue, including the intervertebral discs, was removed, and anatomic evaluation was performed. Ten linear parameters (Figure 5) were measured using a digital ruler (stated accuracy 0.1 mm, inha GmbH, Berlin, Germany). All symmetrical structures were measured bilaterally. Parameters were evaluated relative to a plane defined by the most anteroinferior point of the endplate in the midsagittal plane and the most inferior point of the right and left inferior facet joint surfaces. This scheme was implemented by using a milled plate, which allowed each specimen to rest on these three points.

Statistical Analysis. The accuracy of anatomic, radiographic, and bone mineral density measurements was investigated by repeated measurements. Two vertebrae, two radiographic films, and two CT scans of each species were selected at random to obtain the complete series of measurements performed 10 times each. For comparison between sheep and human parameters, Fisher's least square difference was used. Statistically significant differences were defined at a 95% confidence level. The values are given as mean ± standard deviation (minimum–maximum). Statistical evaluation was supported by SPSS (release 7.5, SPSS Inc. Chicago, IL, USA) software.

■ Results

Radiologic Measurements of Sheep and Human Cervical Spines

Radiologic measurements of linear parameters were accurate to ±0.08 mm. Intervertebral angle and lordosis angle measurements were accurate to ±0.7° and ±1.8°, respectively.

In the human spine, both endplates were concave, whereas in the sheep spine, the upper endplate was convex and the lower endplate concave. Additionally, the curvature of the sheep upper endplates increased in a craniocaudal direction. Therefore, the anterior and mean disc space height (DSH) in the sheep cervical spine in-

Table 1. Intervertebral Disc Space Height (DSH) for Sheep and Human Cervical Spines

	Anterior DSH		Middle DSH		Posterior DSH		Mean DSH	
	Sheep (mm)	Human (mm)	Sheep (mm)	Human (mm)	Sheep (mm)	Human (mm)	Sheep (mm)	Human (mm)
C2–C3	7.0 ± 0.5 (6–8)*	5.4 ± 0.8 (4–8)	6.8 ± 0.5 (5–8)*	5.9 ± 0.7 (4–9)	4.7 ± 0.7 (4–6)	3.9 ± 0.8 (3–5)	6.1 ± 0.7 (5–7.3)*	5.0 ± 0.6 (4–7.5)
C3–C4	7.5 ± 0.4 (7–8)*	5.3 ± 0.5 (4–7)	6.7 ± 0.5 (6–8)	6.2 ± 0.9 (4–8)	4.3 ± 0.5 (4–6)	3.6 ± 0.6 (3–5)	6.2 ± 0.5 (5.3–7)*	5.0 ± 0.7 (3–8)
C4–C5	8.0 ± 0.5 (7–10)*	5.4 ± 0.9 (4–10)	5.9 ± 0.9 (4–7.5)	6.3 ± 1.3 (4–9)	4.4 ± 0.5 (3.5–5)	3.9 ± 0.7 (2–5)	6.1 ± 0.6 (5–6.6)*	5.2 ± 0.6 (4–7)
C5–C6	8.2 ± 0.8 (6–10)*	5.2 ± 1.1 (0.4–1.0)	5.7 ± 0.6 (4–7)	6.5 ± 1.1 (4–8)	4.5 ± 0.6 (4–6)	3.6 ± 0.9 (2–6)	6.1 ± 0.6 (5–7)*	5.1 ± 0.6 (4–7)
C6–C7	8.3 ± 0.9 (7–10)*	5.3 ± 1.2 (4–9)	5.4 ± 0.4 (3.5–6)*	6.4 ± 1.0 (4–9)	4.3 ± 0.4 (3–5)	3.5 ± 0.7 (2–.5)	6.0 ± 0.6 (4–7)*	5.1 ± 0.4 (3–6)

* $P < 0.05$ in comparison with the human spine.

creased constantly from C2–C3 to C6–C7, whereas the middle DSH decreased and the posterior DSH remained unchanged. The anterior and mean DSH were significantly higher in the sheep (Table 1).

In the two species, the intervertebral angles were not significantly different. In both the sheep and human spines, the flexion–extension differences in intervertebral angles increased from C2–C3 to the maximum at C4–C5, then decreased again (Table 2). Functional radiographic evaluation showed significant flexion–extension differences for the lordosis angle between the two species in all motion segments except C5–C6 (Table 3).

Bone Mineral Density Measurements of Sheep and Human Cervical Spines

The BMD measurements were accurate to ±0.008 g/cm^3. Mean BMD values for the human and sheep lower cervical spines are depicted in Table 4.

The standard deviation for BMD of the human cervical spine was fourfold higher than that of the sheep cervical spine. Measurements showed an interlevel BMD variability for the upper and lower endplate in both species, with a maximum at C5 and a minimum at C7. The BMD at the center of the vertebrae increased constantly in a craniocaudal direction. The mean endplate BMD for the sheep and human cervical spines was 32% (C3) to 12% (C7) higher than the BMD at the center of the vertebra ($P < 0.01$). There was no significant difference between the BMDs of the two species in the middle and lower cervical spine.

Stiffness Tests of Sheep and Human Cervical Spines

The results of stiffness tests for the human and sheep cervical spines are depicted in Figures 6 and 7. The range of motion (Figure 6a–c) was significantly different between the two species except for flexion–extension of C3–C4 and C5–C6, axial rotation of C2–C3, and lateral bending of C2–C3, C3–C4, and C4–C5. Stiffness (Figure 7a–c) also was significantly different between the two species except for flexion–extension of C2–C3, C4–C5,

Table 2. Intervertebral Angle for Sheep and Human Cervical Spines in Flexion, Neutral Position, and Extension

	Flexion		Neutral		Extension		Total Motion	
	Sheep (°)	Human (°)	Sheep (°)	Human (°)	Sheep (°)	Human (°)	Sheep (°)	Human (°)
C2–C3	4.6 ± 3.3 (0–8)	4.5 ± 4.0 (0–12)	6.7 ± 3.1 (0–10)	7.5 ± 4.1 (0–16)	7.6 ± 3.8 (0–13)	8.8 ± 4.1 (2–18)	3.0 ± 1.2 (1.1–4.6)	4.3 ± 1.5 (2.7–6.6)
C3–C4	4.5 ± 3.8 (0–11)	4.3 ± 3.3 (0–9)	9.7 ± 3.5 (4–17)	10.3 ± 3.3 (3–19)	12.1 ± 4.0 (5–19)	12.8 ± 4.0 (5–21)	7.6 ± 1.4 (5.8–9.6)	8.5 ± 1.9 (6.3–9.6)
C4–C5	2.6 ± 2.2 (0–6.5)	3.4 ± 2.8 (0–9)	10.8 ± 2.9 (5–16)	11.5 ± 2.7 (6–19)	14.1 ± 4.4 (6–21.5)	14.6 ± 4.1 (7–22)	11.5 ± 1.9 (9.1–13.5)	11.2 ± 1.8 (9.2–13.6)
C5–C6	2.8 ± 2.6 (0–8)	3.6 ± 2.4 (0–10)	10.1 ± 3.4 (6–17)	10.1 ± 2.8 (6–20)	13.8 ± 4.0 (6–18.5)	14.1 ± 4.0 (7–22)	11.0 ± 1.6 (9.0–13.4)	10.5 ± 1.4 (9.0–13.0)
C6–C7	3.8 ± 4.9 (−3–11)	3.7 ± 3.9 (0–10)	9.5 ± 2.1 (5–12)	9.2 ± 3.0 (6–19)	12.6 ± 4.6 (6–20.5)	12.2 ± 4.2 (7–21)	8.8 ± 1.2 (6.8–10.6)	8.5 ± 1.4 (6.1–10.5)

Note: Difference = extension − flexion, − = kyphosis, + = lordosis.

Table 3. Lordosis Angle for Sheep and Human Cervical Spines in Flexion, Neutral Position, and Extension

	Flexion		Neutral		Extension		Total Motion	
	Sheep (°)	Human (°)	Sheep (°)	Human (°)	Sheep (°)	Human (°)	Sheep (°)	Human (°)
C2–C3	−1.2 ± 5.0 (−8–0)	−2.5 ± 4.0 (−6–3)	−1.0 ± 1.5 (−3–0)*	3.1 ± 3.1 (0–11)	3.2 ± 4.7 (−3–7)*	8.2 ± 4.3 (4–18)	4.4 ± 2.7 (1.1–4.6)*	10.7 ± 2.5 (5.1–14.2)
C3–C4	−0.6 ± 4.8 (−8–6)*	−3.1 ± 2.9 (−8–3)	4.7 ± 3.8 (0–11.5)	5.7 ± 3.5 (2–12)	6.7 ± 3.0 (2–12)*	12.4 ± 4.6 (5–21)	7.3 ± 3.4 (5.8–9.6)*	15.5 ± 2.9 (12.3–21.1)
C4–C5	−0.9 ± 4.6 (−8–6)*	−4.8 ± 2.8 (−8–4)	7.6 ± 3.3 (0–12)	7.4 ± 2.9 (2–15)	12.3 ± 1.9 (9.5–14.5)	14.6 ± 5.1 (7–22)	13.2 ± 3.9 (9.1–17.5)*	19.2 ± 3.8 (15.2–24.2)
C5–C6	0.5 ± 6.1 (−6–12)*	−4.8 ± 2.7 (−9–4)	11.0 ± 3.4 (7–17)	9.1 ± 3.8 (3–20)	18.4 ± 3.4 (12.5–24)	15.7 ± 4.9 (7–22)	17.9 ± 3.6 (13.0–22.4)	20.5 ± 3.4 (16.0–23.4)
C6–C7	5.2 ± 5.5 (0–13)*	−3.4 ± 3.1 (−7–3)	22.8 ± 3.8 (18–29)*	8.3 ± 3.0 (3–19)	28.8 ± 5.6 (18.5–36)*	15.1 ± 4.5 (7–21)	23.6 ± 4.2 (18.8–28.0)*	18.5 ± 3.4 (14.0–23.6)

Note: Difference = extension − flexion, − = kyphosis, + = lordosis.
* $P < 0.05$ in comparison with the human spine.

Table 4. Bone Mineral Density (BMD) of Sheep and Human Cervical Spines

	C3		C4		C5		C6		C7	
	Sheep (g/cm^3)	Human (g/cm^3)	Sheep (g/cm^3)	Human (g/cm^3)	Sheep (g/cm^3)	Human (g/cm^3)	Sheep (g/cm^3)	Human (g/cm^3)	Sheep (g/cm^3)	Human (g/cm^3)
Upper endplate	0.452 ± 0.031	0.417 ± 0.124	0.456 ± 0.034	0.442 ± 0.142	0.468 ± 0.030	0.447 ± 0.112	0.450 ± 0.029	0.426 ± 0.152	0.431 ± 0.028	0.399 ± 0.119
Center	0.341 ± 0.026	0.312 ± 0.102	0.338 ± 0.025	0.333 ± 0.098	0.360 ± 0.031	0.343 ± 0.118	0.372 ± 0.021	0.319 ± 0.118	0.382 ± 0.022	0.304 ± 0.089
Lower endplate	0.460 ± 0.028	0.446 ± 0.108	0.460 ± 0.032	0.464 ± 0.142	0.472 ± 0.041	0.464 ± 0.102	0.455 ± 0.020	0.414 ± 0.134	0.439 ± 0.033	0.402 ± 0.107

and C5–C6, and lateral bending of C2–C3, C3–C4, and C4–C5.

Anatomic Measurements of Sheep and Human Cervical Spines

Anatomic measurements were accurate to ±0.08 mm. The results of anatomic measurements are depicted in Table 5. In the sheep cervical spine, anterior vertebral body height typically was higher than posterior vertebral body height, whereas these parameters were not different in the human spine. Sheep vertebrae were conical, whereas human vertebrae were cylindrical. As a result, the lower endplate parameters (lower endplate width and depth) for the sheep spine were constantly greater than upper endplate parameters (upper endplate width and depth). Therefore, a significant difference in lower endplate parameters for the two species was observed. Yet, no difference was found in upper endplate parameters for C4 and C5.

All the sheep vertebrae were taller in size (1.4- to 2.8-fold) than the human vertebrae. The sheep pedicles were taller than the human pedicles (4.5- to 2.1-fold), but no difference was found in pedicle width. Spinal canal parameters (spinal canal depth and width) were always higher in the human spine.

■ Discussion

Although the sheep cervical spine has been used repeatedly as a model in spine research, little quantitative data are available.[34,35] Therefore, the aim of this study was to determine quantitative anatomic, radiographic, computerized tomographic, and biomechanical data of the sheep cervical spine and compare it directly with the corresponding data of the human spine.

Radiographic evaluation showed an average DSH of 6 mm in the sheep cervical spine, which was approximately 1 mm (15%) higher than the DSH in the human cervical spine. In contrast, Wilke et al[35] assumed a difference of 2 to 3 mm, citing the data of Moroney et al.[22] In the lumbar spine, differences in intervertebral DSH between sheep and humans are substantially higher. Anterior DSH in the human lumbar spine ranges between 11 and 16 mm,[10,23] whereas that of sheep shows a mean DSH of 4.2 to 4.5 mm.[35] Therefore, with regard to DSH, the sheep cervical spine may represent a better model for human studies than the sheep lumbar spine.

Functional radiographic assessment (flexion–extension radiographs) often is applied clinically to evaluate interbody fusion.[2,17,28] In the current study, the intervertebral angles of the two species were not significantly different. Similarities in at least major dimensions were observed also for the lordosis angle, especially for the motion segment C5–C6. Therefore, if fusion is the goal of an animal study, the functional radiographic data of the normal sheep cervical spine motion segment reported in this discussion may be a helpful tool for distinguishing among normal, fused (without any motion), and nonfused motion segments.

To the authors' knowledge, BMD data for the sheep cervical spine do not exist, and only one available study addresses BMD variations of the human cervical spine. Curylo et al[5] studied 17 human cadaver lower cervical spines (C4–C7) using dual-energy radiograph absorptiometry and showed higher BMD values than those obtained in the current study using quantitative CT scans.

In accordance with Curylo et al,[5] the current study showed a significant variability in cervical spine interlevel BMD, suggesting that random single-segment bone mineral density measurements or mean specimen bone mineral density values may not be relevant for predicting BMD in this specific region. In the current study, no significant difference was found between the BMD values of human and sheep lower cervical spines. The least percentile difference between the two species was found for C4. Standard deviations for the BMD of the human cervical spine were fourfold higher than the BMD of the sheep cervical spine. This may be important for the testing of fixation devices such as plates, screws, and interbody fusion cages because a quasi–BMD-independent evaluation of human implants is possible using the sheep cervical spine model.

Many *in vivo* and *in vitro* experiments have relied on the sheep spine model, although there was little knowledge about biomechanical comparability with the human spine.[1,11,16,20,21,24,25,36] Many of these studies were *in vivo* investigations to evaluate the results of disc surgery.[1,11,20,21,25] Wilke et al[34] was the first to describe biomechanical analogies between sheep and the human spines by testing sheep spines and comparing the data to the literature. Although many data are available from *in vitro* and *in vivo* tests of the normal human cervical

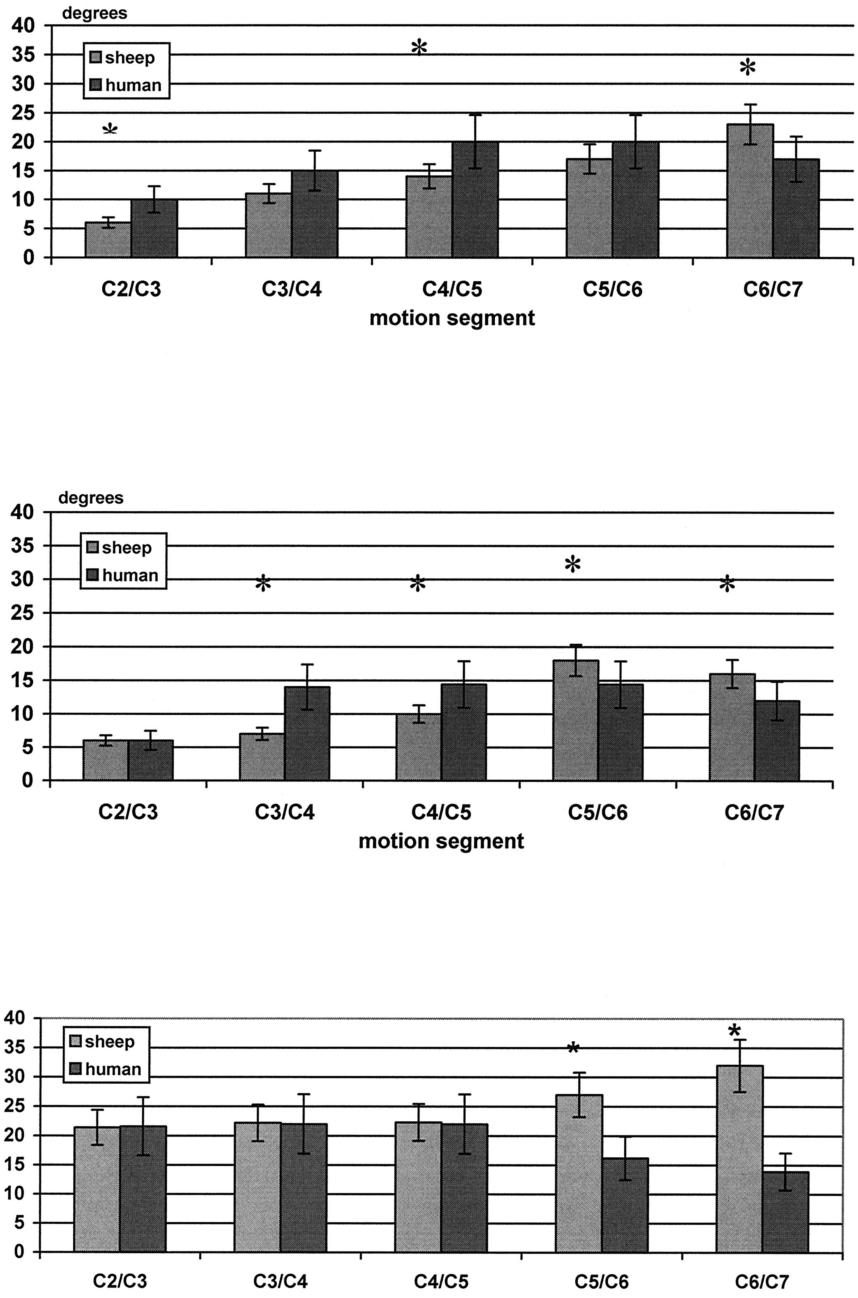


Figure 6. Range of motion at 6 Nm in human and sheep cervical spine specimens. **a,**Combined segmental flexion–extension range of motion in the human and sheep cervical spine. $*P < 0.05$ in comparison with the human spine.

spine,[19,32,33] comparison remains difficult because of the large variety in test setups.

The current study is the first to compare sheep and human cervical spines biomechanically with an identical test setup. The range of motion results for the sheep cervical spine in this study were slightly higher than those previously reported by Wilke et al,[34] most likely because of the higher moments applied (2.5 *vs* 6 Nm). Although range of motion and stiffness data were mainly comparable in their craniocaudal trends-,significant differences between the two species were observed, especially for rotation parameters. If the sheep model is used, the motion segments C2–C3 and C3–C4 appear to be the most suitable for biomechanical tests.

Anatomic evaluation of the human cervical spine showed values comparable with those of previous studies.[6,9,14,26,27,29,31] A number of fundamental anatomic differences in the cervical spine were observed between the two species: Sheep vertebrae were taller than wide, whereas human vertebrae were wider than tall; sheep vertebral bodies had a conic shape, whereas human vertebral bodies were cylindrical; sheep pedicles were ellipsoid, whereas human pedicles were nearly round; and in the sheep spine, the upper endplate was convex and the lower endplate concave, whereas both endplates were concave in the human spine. Yet, fundamental similarities also existed between the two species. In particular, no significant difference could be found between the upper endplate

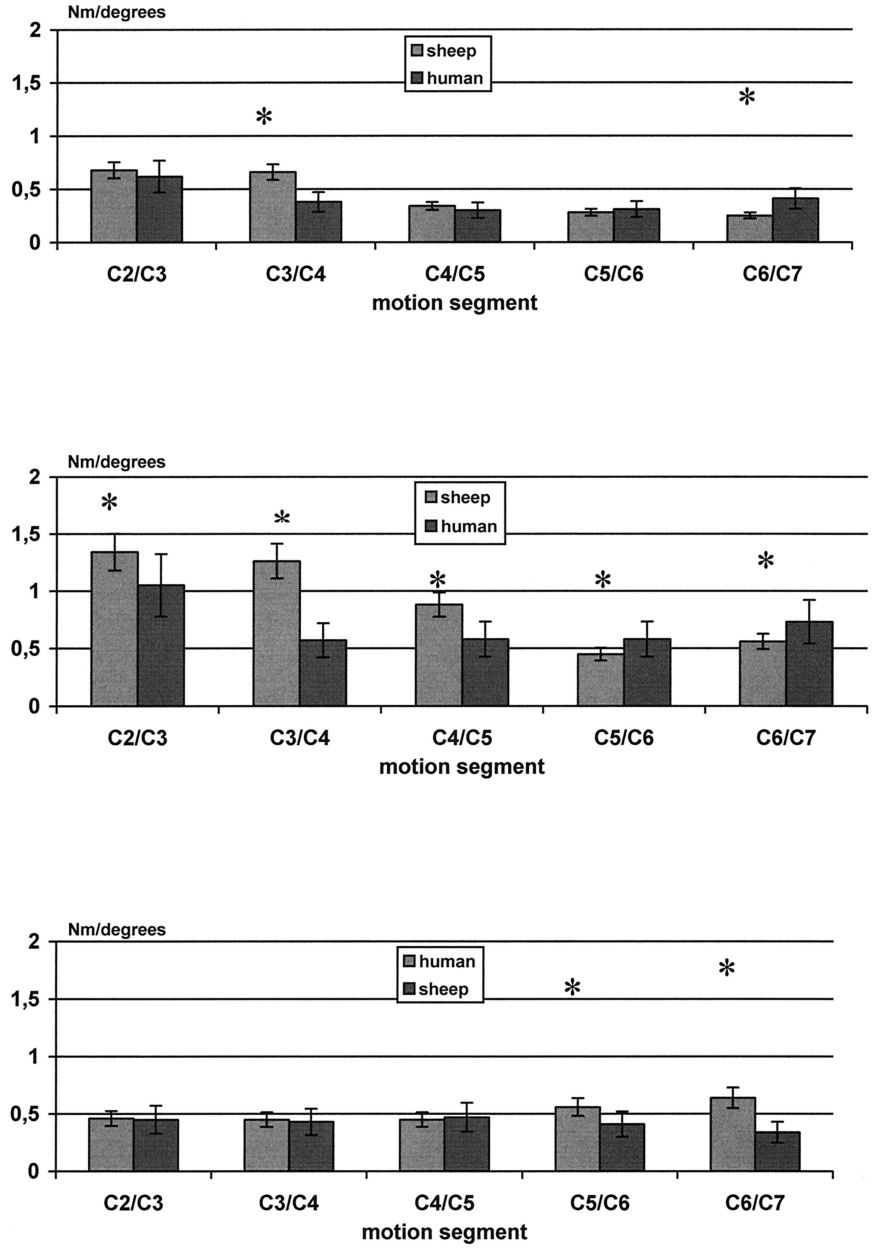


Figure 7. Mean apparent stiffness in human and sheep cervical spine specimens. **a,** Flexion–extension stiffness in the human and sheep cervical spine. *$P <$ 0.05 in comparison with the human spine.

parameters (upper endplate depth and width) for C4 and C5.

Without doubt, sheep and human cervical spines are different, both in appearance and function. The statistical evaluation in this study confirmed this difference. But statistics also highlighted the fact that the motion segment C3–C4 showed the least difference, as compared with the corresponding human motion segment. Although lower endplate depth and width parameters in this motion segment are significantly different, upper endplate depth and width as well as DSH parameters are similar enough to allow implantation of human cervical spine interbody implants. If the object is the testing of human cervical spine interbody implants, the authors think that the sheep motion segment C3–C4 is a valid model, both in appearance and function. For other research purposes species-specific cages, taking the various lower endplate depth and width parameters into account might be more suitable.

■ Conclusion

The results of this study provide a comprehensive morphologic and biomechanical database on the 2-year-old female Merino sheep cervical spine. Although there were distinct differences between the human and sheep cervical spines, the small intergroup standard deviations and the good comparability with the human spine encourage the use of the sheep cervical spine as a model for human cervical spine research. On the basis of proven analogy, the sheep motion segment

Table 5. Anatomic Parameters of Sheep and Human Cervical Spines

	C3		C4		C5		C6		C7	
	Sheep (mm)	Human (mm)	Sheep (mm)	Human (mm)	Sheep (mm)	Human (mm)	Sheep (mm)	Human (mm)	Sheep (mm)	Human (mm)
AVBH	45.6 ± 0.82 (41–46)*	15.3 ± 1.56 (11–18)	44.3 ± 0.79 (42–47)*	14.5 ± 1.14 (11–18)	40.3 ± 0.87 (37–43)*	14.2 ± 0.99 (11–17)	35.1 ± 1.01 (31–38)*	14.4 ± 1.21 (11–18)	27.6 ± 1.06 (22–29)*	15.1 ± 1.18 (11–18)
PVBH	44.5 ± 0.92 (40–46)*	15.4 ± 1.14 (12–19)	42.4 ± 0.88 (42–47)*	14.8 ± 1.27 (10–19)	39.5 ± 0.92 (36–44)*	14.7 ± 1.08 (10–17)	34.4 ± 0.81 (30–37)*	14.3 ± 1.21 (10–16)	27.0 ± 0.93 (24–30)*	15.3 ± 1.28 (12–19)
UEPD	20.0 ± 0.66 (17–23)*	17.7 ± 1.04 (14–20)	19.2 ± 0.71 (18–22)	18.2 ± 1.43 (14–23)	19.6 ± 0.77 (18–23)	18.4 ± 1.21 (15–23)	20.2 ± 0.61 (18–23)	18.6 ± 1.34 (14–24)	20.8 ± 0.68 (18–22)	19.2 ± 1.29 (16–24)
UEPW	24.7 ± 0.81 (21–28)*	20.1 ± 1.10 (17–25)	25.4 ± 0.56 (24–27)	23.8 ± 1.33 (19–27)	24.6 ± 0.59 (22–27)	24.9 ± 1.23 (21–28)	22.9 ± 0.64 (22–27)*	27.0 ± 1.22 (22–31)	21.4 ± 0.63 (19–24)*	29.3 ± 1.56 (25–34)
LEPD	25.8 ± 0.62 (22–27)*	17.6 ± 1.43 (14–21)	26.5 ± 0.61 (25–29)*	18.3 ± 1.34 (14–22)	23.9 ± 0.67 (22–27)*	18.6 ± 1.48 (14–24)	22.9 ± 0.55 (21–26)*	19.3 ± 1.29 (16–24)	21.0 ± 0.51 (19–23)	20.0 ± 1.34 (16–24)
LEPW	29.2 ± 0.71 (26–33)*	20.6 ± 1.20 (17–25)	29.1 ± 0.69 (27–32)*	22.4 ± 0.98 (19–27)	28.5 ± 0.80 (27–33)*	24.0 ± 1.56 (18–28)	25.9 ± 0.76 (23–29)	27.0 ± 1.38 (23–31)	24.4 ± 0.83 (22–28)*	29.4 ± 1.43 (24–32)
SCD	10.9 ± 0.45 (9–13)*	16.5 ± 0.98 (14–18)	11.6 ± 0.38 (10–13)*	15.4 ± 1.22 (13–19)	12.3 ± 0.39 (10–14)*	15.1 ± 1.07 (12–18)	14.1 ± 0.42 (12–15)	15.4 ± 0.89 (11–18)	13.8 ± 0.44 (12–16)	15.9 ± 1.23 (12–19)
SCW	15.2 ± 0.59 (12–17)*	24.6 ± 1.23 (20–28)	15.8 ± 0.52 (14–18)*	25.7 ± 1.56 (20–31)	15.9 ± 0.63 (14–17)*	25.6 ± 1.43 (20–30)	16.9 ± 0.23 (15–19)*	25.9 ± 1.34 (22–29)	17.9 ± 0.35 (16–20)*	24.6 ± 1.11 (20–28)
PH	32.3 ± 1.02 (27–37)*	7.4 ± 1.09 (6–10)	30.2 ± 1.11 (27–34)*	7.6 ± 1.22 (5–11)	26.4 ± 1.03 (24–31)*	7.7 ± 0.89 (6–10)	21.1 ± 0.94 (20–26)*	8.1 ± 0.72 (6–11)	17.3 ± 0.77 (14–19)*	8.5 ± 0.73 (6–11)
PW	4.7 ± 0.31 (4–6)	5.0 ± 0.78 (3–8)	4.4 ± 0.42 (3–6)	5.3 ± 0.72 (3–8)	5.0 ± 0.37 (4–6)	5.8 ± 0.58 (3–8)	6.2 ± 0.43 (4–7)	6.2 ± 0.56 (4–10)	14.7 ± 0.39 (11–17)*	7.3 ± 0.53 (4–11)

* $P < 0.05$ in comparison with the human spine.
AVBH = anterior vertebral body height, PVBH = posterior vertebral body height, UEPD = upper endplate depth, UEPW = upper endplate width, LEPD = lower endplate depth, LEPW = lower endplate width, SCD = spinal canal depth, SCW = spinal canal width, PH = pedicle height, PW = pedicle width.

C3–C4 especially seemed to represent a valid model for corresponding human motion segment.

References

1. Ahlgren BD, Vasavada MS, Brower RS, et al. Anular incision technique on the strength and multidirectional flexibility of the healing intervertebral disc. Spine 1994;19:948–54.
2. Brantigan JW. Pseudarthrosis rate after allograft posterior lumbar interbody fusion with pedicle screw and plate fixation. Spine 1994;19:1271–80.
3. Cain CMJ, Fraser RD. Bony and vascular anatomy of the normal cervical spine in the sheep. Spine 1995;20:759–65.
4. Crawford NR, Brantley AGU, Dickman CA, et al. An apparatus for applying pure nonconstraining moments to spine segments *in vitro*. Spine 1995;20: 2097–2100.
5. Curylo LJ, Lindsey RW, Doherty BJ, et al. Segmental variations of bone mineral density in the cervical spine. Spine 1996;21:319–22.
6. Ebraheim NA, Rongming X, Knight T, et al. Morphometric evaluation of lower cervical pedicle and its projection. Spine 1997;22:1–6.
7. Edmonston SJ, Singer KP, Day RE, et al. Formalin fixation effects on vertebral bone density and failure mechanics: A study of human and sheep vertebra. Clin Biomech 1994;9:175–9.
8. Eggli S, Schläpfer F, Angst M, et al. Biomechanical testing of three newly developed transpedicular multisegmental fixation systems. Eur Spine J 1992; 1:109–16.
9. Francis CC. Dimensions of cervical vertebrae. Anat Rec 1955;122:603–9.
10. Green TP, Adams MA, Dolan P. Tensile properties of the anulus fibrosus: Ultimate tensile strength and fatigue life. Eur Spine J 1993;2:209–14.
11. Gunzburg R, Fraser RD, Moore R, et al. An experimental study comparing percutaneous discectomy with chemonucleolysis. Spine 1993;18: 218–26.
12. Gurwitz GGS, Dawson JM, McNamara MJ, et al. Biomechanical analysis of three surgical approaches for lumbar burst fractures using short-segment instrumentation. Spine 1993;18:977–82.
13. Kandziora F, Kerschbaumer F, Starker M, et al. Biomechanical assessment of the transoral plate fixation for atlantoaxial instability. Spine 2000;25:1555–61.
14. Karaikovic EE, Daubs, MD, Madsen RW, et al. Morphometric characteristics of human cervical pedicles. Spine 1997;22:493–500.
15. Kleemann R. Entwicklung eines Wirbelsäulenprüfstands zur Testung von Implantaten an der Halswirbelsäule: Semesterarbeit. Fakultäat für Maschinenbau. Berlin, Germany: Technische Universität Berlin, 1999.
16. Kotani Y, Cunningham BW, Cappucino A, et al. The role of spinal instrumentation on augmenting posterolateral fusion. Spine 1996;21: 278–87.
17. Kuslich SD, Ulstrom CL, Griffith SL, et al. The Bagby and Kuslich method of lumbar interbody fusion: History, techniques, and 2-year follow-up of a United States prospective, multicenter trial. Spine 1998;23:1267–79.
18. Lee EJ, Hung YC, Lee MY, et al. Kinematics of cervical spine discectomy with and without bone grafting: Quantitative evaluation of late fusion in a sheep model. Neurosurgery 1999;44:139–46.
19. McClure P, Siegler S, Nobilini R. Three-dimensional flexibility characteristics of the human cervical spine *in vivo*. Spine 1998;23:216–23.
20. Melrose J, Ghosh P, Taylor TKF, et al. A longitudinal study of the matrix changes induced in the intervertebral discs by surgical damage to the annulus fibrosus. J Orthop Res 1992;10:665–76.
21. Moore RJ, Osti OL, Vernon-Roberts B, et al. Changes in endplate vascularity after an outer anulus tear in the sheep. Spine 1992;17:874–8.
22. Moroney SP, Schultz AB, Miller JAA, et al. Load displacement properties of lower cervical spine motion segments. J Biomech 1988;21:769–79.
23. Nachemson A, Schiltz AB, Berkson MH. Mechanical properties of human lumbar spine motion segments: Influence of age, sex, disc level, and degeneration. Spine 1979;4:1–8.
24. Nagel DA, Kramers PC, Rahn BA, et al. A paradigm of delayed union and nonunion in the lumbosacral joint: A study of motion and bone grafting of the lumbosacral spine in sheep. Spine 1991;16:553–9.
25. Osti OL, Vernon-Roberts B, Fraser RD. Anulus tear and intervertebral disc degeneration: An experimental study using an animal model. Spine 1990;15: 431–5.
26. Pait GT, Killefer JA, Arnautovic KI. Surgical anatomy of the anterior cervical spine: The disc space, vertebral artery, and associated bony structures. Neurosurgery 1996;39:769–76.
27. Panjabi MM, Duranceau J, Goel V, et al. Cervical human vertebrae: Quantitative three-dimensional anatomy of the middle and lower regions. Spine 1991;16:861–9.
28. Ray CD. Threaded fusion cages for lumbar interbody fusions: An economic comparison with 360 degrees fusion. Spine 1997;22:681–5.

29. Rongming X, Kang A, Ebraheim NA, et al. Anatomic relation between the cervical pedicle and the adjacent neural structures. Spine 1999;24:451–4.
30. Slater R, Nagel R, Smith RL. Biochemistry of fusion mass consolidation in the sheep spine. J Orthop Res 1988;6:138–44.
31. Tominaga T, Dickmann CA, Sonntag VKH, et al. Comparative anatomy of the baboon and human cervical spine. Spine 1995;20:131–7.
32. Voo LM, Pintar FA, Yoganandan N, et al. Statis and dynamic bending response of the human cervical spine. J Biomech Eng 1998;120:693–6.
33. Wen N, Lavaste F, Santin JJ, et al. Three-dimensional biomechanical properties of the human spine *in vitro*. Eur Spine J 1993;2:2–47.
34. Wilke HJ, Kettler A, Claes LE. Are sheep spines a valid biomechanical model for human spines? Spine 1997;22:2365–74.
35. Wilke HJ, Kettler A, Wenger KH, et al. Anatomy of the sheep spine and its comparison to the human spine. Anat Rec 1997;247:542–55.
36. Yamamuro T, Shikata J, Okumura H, et al. Replacement of the lumbar vertebrae of sheep with ceramic prostheses. J Bone Joint Surg [Br] 1990;72:889–93.

Address reprint requests to

Frank Kandziora, MD
Unfall-und
Wiederherstellungschirurgie
Universitätsklinikum Charité der Humboldt
Universität Berlin
Campus Virchow Klinikum
Augustenburgerplatz 1
13353 Berlin
Germany
E-mail: frank.kandziora@charite.de

Da sich in dieser Untersuchung gezeigt hatte, dass das Bewegungssegment C3/C4 der Schafshalswirbelsäule als Model des humanen Bewegungssegmentes C3/C4 geeignet ist, wurden alle weiteren Untersuchungen an diesem Bewegungssegment durchgeführt.

2.2 Zervikale Cages

2.2.1 In vitro Untersuchung zervikaler Cages

Zervikale Cages können nach Weiner [200] in 3 verschiedene Designgruppen unterteilt werden: Schrauben-, Box- und Zylinderdesigne. Die bisher vorliegenden Untersuchungen [140,203] lassen keine eindeutige Aussage darüber zu, ob prinzipielle Unterschiede in der biomechanischen in vitro Primärstabilität zwischen diesen Cagedesigngruppen bestehen. Der Einfluss des Cagedesigns auf die biomechanische in vitro Primärstabilität bleibt demzufolge momentan unklar und sollte in dieser rein biomechanischen Untersuchung geklärt werden.

- Daher sollten in dieser Untersuchung Cages aller drei Designgruppen hinsichtlich ihrer biomechanischen Kenngrößen Bewegungsumfang, Steifigkeit, neutrale und elastische Zone verglichen werden, um potentielle Unterschiede zwischen den Designgruppen zu evaluieren.
- Zusätzlich sollten Parameter entwickelt werden, die es erlauben, anhand von biomechanischen in vitro Daten die in vivo Einheilung eines Cages zu prognostizieren.
- Schließlich sollten anhand der gewonnenen Daten Cages ausgewählt werden, die für eine in vivo Evaluation im Tiermodell geeignet sind.

SPINE Volume 26, Number 17, pp 1850–1857

Biomechanical Comparison of Cervical Spine Interbody Fusion Cages

Frank Kandziora, MD,* Robert Pflugmacher,* Jan Schäfer,* Christian Born, MD,† Georg Duda, PhD,* Norbert P. Haas, MD,* and Thomas Mittlmeier, MD*

Study Design. An *in vitro* biomechanical study of cervical spine interbody fusion cages using a sheep model was conducted.

Objectives. To evaluate the biomechanical effects of cervical spine interbody fusion cages, and to compare three different cage design groups.

Summary and Background Data. Recently, there has been a rapid increase in the use of cervical spine interbody fusion cages as an adjunct to spondylodesis. These cages can be classified into three design groups: screw, box, or cylinder designs. Although several comparative biomechanical studies of lumbar interbody fusion cages are available, biomechanical data for cervical spine constructs are lacking. Additionally, only limited data are available concerning comparative evaluation of different cage designs.

Methods. In this study, 80 sheep cervical spines (C2–C5) were tested in flexion, extension, axial rotation, and lateral bending with a nondestructive stiffness method using a nonconstrained testing apparatus. Three-dimensional displacement was measured using an optical measurement system (Qualysis). Complete discectomy (C3–C4) was performed. Cervical spine interbody fusion cages were implanted according to manufacturers' information. Eight spines in each of the the following groups were tested: intact, autologous iliac bone graft, two titanium screws (Novus CTTi; Sofamor Danek, Koln, Germany), two titanium screws (BAK-C 8 mm; Sulzer Orthopedics, Baar, Switzerland), one titanium screw (BAK-C 12 mm; Sulzer Orthopedics), carbon box (Novus CSRC; Sofamor Danek), titanium box (Syncage; Synthes, Bochum, Germany), titanium mesh cylinder (Harms; DePuy Acromed, Sulzbach, Germany), titanium cylinder (MSD; Ulrich, Ulm, Germany), and titanium cylinder (Kaden; BiometMerck, Berlin, Germany). The mean apparent stiffness values were calculated from the corresponding load-displacement curves. Additionally, cage volume and volume-related stiffness was determined.

Results. After cervical spine interbody fusion cage implantation, flexion stiffness increased, as compared with that of the intact motion segment. On the contrary, rotation stiffness decreased after implantation of a cervical spine interbody fusion cage, except for the Novus CSRC, Syncage, and Kaden-Cage. If two screws were inserted (Novus CTTi and BAK-C 8 mm), there was no significant difference in flexion stiffness between screw and cylinder design groups. If one screw was inserted (BAK-C 12 mm), flexion stiffness was higher for cylinder designs ($P < 0.05$). Extension and bending stiffness were always higher with cylinder designs ($P < 0.05$). Volume-related stiffness-for flexion extension and bending was highest for the Harms cage ($P < 0.05$). There was no difference for rotation volume-related stiffness between Harms and Syncage.

Conclusions. The biomechanical results indicate that design variations in screw and cylinder design groups are of little importance. In this study, however, cages with a cylinder design were able to control extension and bending more effectively than cages with a screw design. [Key words: animal model, biomechanics, cages, cervical spine, sheep] **Spine 2001;26:1850–1857**

Anterior decompression and interbody fusion are widely accepted as surgical treatment for patients with cervical spondylosis. Tricortical iliac crest bone graft, the gold standard heretofore, is associated with high donor site morbidity. Additional problems such as pseudarthrosis, graft collapse with kyphotic deformity, and graft extrusion have led to a rapid increase in the use of cervical spine interbody fusion cages (CSIFC) as an adjunct to spondylodesis.[3,19,22,23,26,43] Yet, biomechanical data are lacking.

Interbody fusion cages are being developed in the quest for interspace structural stability during bony fusion. They have been promoted with the aim to provide immediate strong anterior column support. Several interbody construct designs have been developed. According to Weiner and Fraser,[43] these construct designs can be subdivided into three design groups: screw (horizontal cylinder), box, or cylinder (vertical ring) designs. Although several comparative biomechanical studies of lumbar interbody fusion cages are available,[2,4,9,10,12,16,21,28,32–34,40,41,50] cervical spine constructs have not yet been addressed. Additionally, only little biomechanical data are available concerning comparative evaluation of different cage designs.

Therefore, the aims of this study were to evaluate biomechanical properties of cervical spine interbody fusion cages, and to compare three CSIFC design-groups.

■ Material and Methods

Spine Preparation. In this study, 80 cervical spines (C2–C5) of 2-year-old adult female Merino sheep (average weight, 67.2 ± 4.6 kg) were used for biomechanical testing. *En bloc* specimens were stored at −20 C until they were thawed in a water bath at 25 C. The motion segment C3–C4 was isolated, and superficial musculature was removed. Care was taken to preserve all ligaments. Each specimen was radiographically screened to exclude abnormalities that might compromise the mechanical properties of the sheep cervical spine.

To simulate essential clinical features, a complete discectomy of C3–C4 with resection of the anterior longitudinal ligament was performed. The endplates were shaved using a high-

From the *Unfall- und Wiederherstellungschirurgie, and the †Strahlenklinik und Poliklinik Universitätsklinikum Charité der Humboldt Universität Berlin, Campus Virchow-Klinikum, Berlin, Germany.
Acknowledgment date: June 9, 2000.
Acceptance date: December 22, 2000.
Device status category: 7,11.
Conflict of interest category: 12.

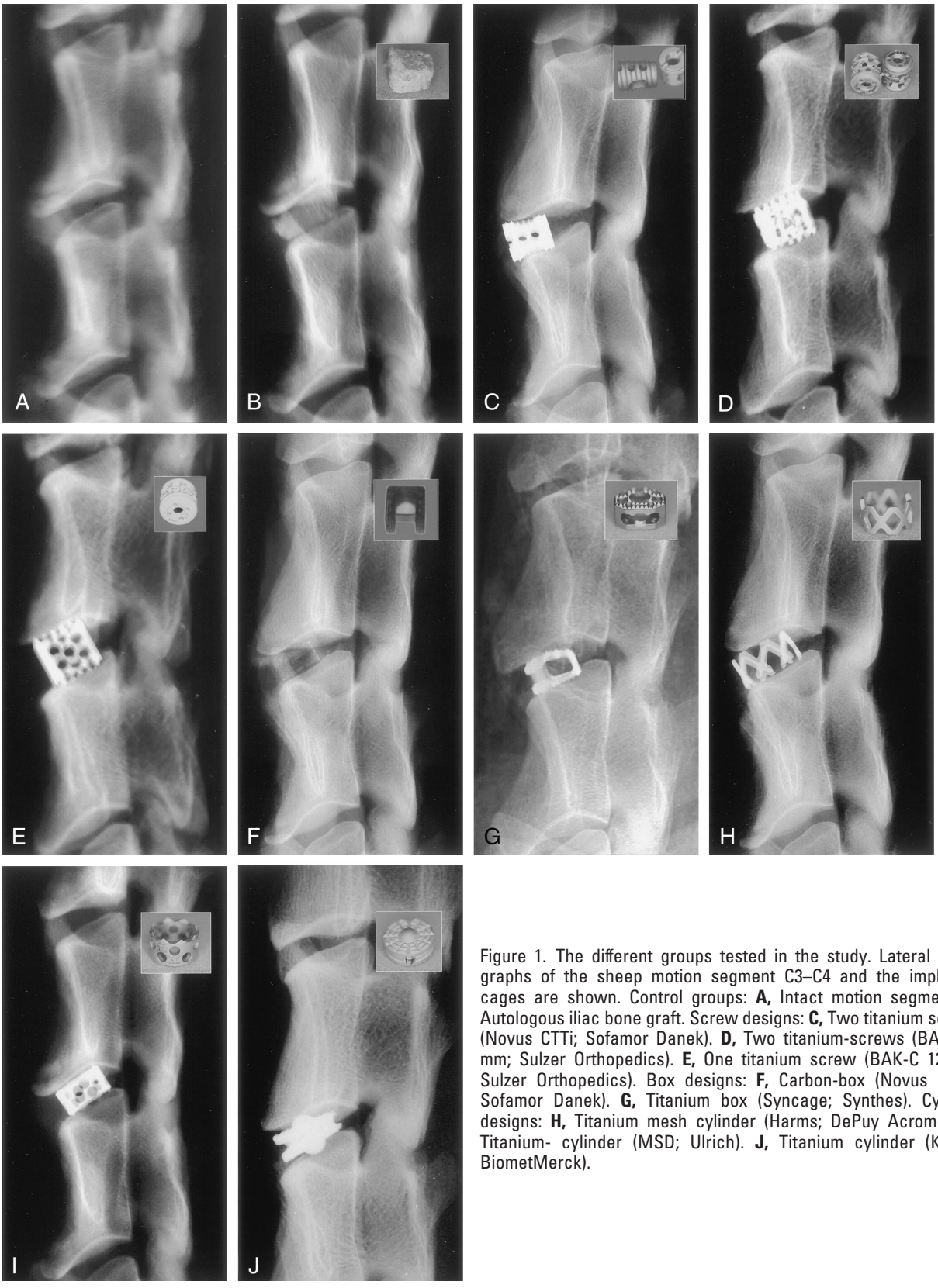


Figure 1. The different groups tested in the study. Lateral radiographs of the sheep motion segment C3–C4 and the implanted cages are shown. Control groups: **A,** Intact motion segment. **B,** Autologous iliac bone graft. Screw designs: **C,** Two titanium screws (Novus CTTi; Sofamor Danek). **D,** Two titanium-screws (BAK-C 8 mm; Sulzer Orthopedics). **E,** One titanium screw (BAK-C 12 mm, Sulzer Orthopedics). Box designs: **F,** Carbon-box (Novus CSRC; Sofamor Danek). **G,** Titanium box (Syncage; Synthes). Cylinder designs: **H,** Titanium mesh cylinder (Harms; DePuy Acromed). **I,** Titanium- cylinder (MSD; Ulrich). **J,** Titanium cylinder (Kaden; BiometMerck).

speed diamond burr. Cervical interbody fusion cages were implanted according to manufacturers' information.

Cervical Spine Interbody Fusion Cages. Cages are shown in Figure 1. The height, width, and depth of the CSIFCs used in this study are depicted in Table 1. To allow comparison between the different cage designs, cages of similar height, width, and depth were used.

The volume of the cages was determined by the water displacement technique, according to the Archimedes' principle.[11]

Table 1. Height, Width, and Depth of the Cervical Spine Interbody Fusion Cages

Group	Cage	Type	Company	Material	Height (mm)	Width (mm)	Depth (mm)
2	Iliac bone graft	—	—	Bone	8	14	14
3	Novus CTTi*	Screw	Sofamor Danek	Titanium	8	8	12
4	BAK-C*	Screw	SulzerMedica	Titanium	8	8	12
5	BAK-C	Screw	SulzerMedica	Titanium	12	12	12
6	Novus CRSC	Box	Sofamor Danek	Carbon	7	8	12
7	Syncage C	Box	Synthes	Titanium	7	15	13
8	Harms	Cylinder	DePuyAcroMed	Titanium	8	14	14
9	MSD	Cylinder	Ulrich	Titanium	8	14	14
10	Kaden	Cylinder	BiometMerck	Titanium	7	16	15

* The producer recommends the use of two parallel inserted cages of this height for cervical interbody fusion.

To measure endplate implant contact area, the cages were cut in two horizontal parts. Standardized axial digital pictures were taken for each upper and lower part of the cages. The endplate implant contact area was measured using digital picture analysis system (Kontron; Zeiss, Jena, Germany).

Test Setup. Testing was performed by a nondestructive flexibility method using a nonconstrained testing apparatus described elsewhere in detail.[13,15] Pure bending moments were applied using a system of cables and pulleys to induce flexion, extension, left and right lateral bending, and left and right axial rotation, correspondingly. Tension was applied to the cables with a uniaxial testing machine (Zwick 1456; Zwick GmbH, Ulm, Germany). Applied forces were measured with an axial load cell (Z 12; HBM, Darmstadt, Germany) mounted on the testing frame. Moments were calculated by multiplying the applied force by the radius of the pulley on the spine-testing fixture.

Three-dimensional displacement of each motion segment was measured using an optical measurement system (Qualysis, Sävebalden, Sweden). Nonlinear diodes (Qualysis) were attached to the corpora of C3 and C4. Marker positions were detected with two cameras and recorded with a computerized motion analysis system (PC-Reflex; Qualysis). Angular displacement of the upper vertebra in relation to the lower vertebra was calculated from a marker position using custom-made computer software. The experimental error associated with this method was ±0.12°.

Study Protocol. Eight spines were assigned randomly to be tested in each of the following groups: intact, autologous tricortical iliac bone graft, two titanium screws (Novus CTTi; Sofamor Danek, Koln, Germany), two titanium screws (BAK-C 8 mm; Sulzer Orthopedics, Baar, Switzerland), one titanium screw (BAK-C 12 mm; Sulzer Orthopedics), carbon box (Novus CSRC; Sofamor Danek), titanium box (Syncage; Synthes, Bochum, Germany), titanium mesh cylinder (Harms; DePuy Acromed, Sulzbach, Germany), titanium cylinder (MSD; Ulrich, Ulm, Germany), and titanium cylinder (Kaden; BiometMerck, Berlin, Germany).

Specimens were kept moist during tests. For testing, C2 and C5 were mounted in pots using polymethylmethacrylate (Technovit 3040; Heraeus Kulzer GmbH, Wehrheim/Ts, Germany). The lower pot was attached rigidly to the base of the testing apparatus. This test setup resulted in a compressive preload of 25 N from the weight of the upper fixation pot, which represents the average weight of the sheep head. Moments were applied in a quasistatic manner in increments of 1 to 6 Nm. At each step, to minimize viscoelastic response, the specimen was allowed to relax for 60 seconds before data were recorded. Test modes were flexion, extension, left and right axial rotation, and left and right lateral bending. Specimens were preconditioned using three cycles of 6-Nm load with a 1.2-mm/second velocity of the traverse bar. The fourth cycle was measured. The mean apparent stiffness values in the elastic zone were calculated from the corresponding load-displacement curves. Volume-related stiffness was determined by dividing stiffness results by cage volume.

Statistical Analysis. Statistical analysis was performed using the Mann-Whitney *U* test and Wilcoxon's rank sum test. Statistical significant differences were defined at a 95% confidence level. The values are given as mean ± standard deviation. Statistical evaluation was supported by SPSS (release 7.5, SPSS; Chicago, IL) software.

■ Results

Volume of Cages and Endplate Implant Contact Area

The results of volume and endplate implant contact area measurements are depicted in Table 2.

Stiffness Tests

Figure 2 summarizes the stiffness of the CSIFCs normalized with respect to the intact motion segment during flexion, extension, rotation, and bending.

Comparison Between Cages and Intact Motion Segment

Screw Designs. In comparison with the intact motion segment, all the cages with screw designs were able to stabilize the motion segment during flexion ($P < 0.01$). There was no significant difference in stiffness between the Novus CTTi and the intact motion segment in extension, rotation, and bending. The BAK-C cages (8 and 12 mm) were not able to restore stiffness of the intact motion segment in extension, rotation, and bending ($P < 0.05$).

Box Designs. In comparison with the intact motion segment, the flexion stiffness of the Novus CSRC was significantly greater ($P < 0.05$). No significant difference was found in the stiffness between the Novus CSRC and the intact motion segment in extension, rotation, and bending. In comparison with the intact motion, the seg-

Table 2. Results From Volume Measurements of the Cervical Spine Interbody Fusion Cages

Group	Cage	Type	Company	Volume (cm^3)	uEICA (cm^2)	lEICA (cm^2)
2	Iliac bone graft	—	—	1.32	—	—
3	Novus CTTi*	Screw	Sofamor Danek	0.58 (2 screws)*	0.23	0.23
4	BAK-C 8 mm*	Screw	SulzerMedica	0.56 (2 screws)*	0.25	0.25
5	BAK-C 12 mm	Screw	SulzerMedica	0.38	0.30	0.30
6	Novus CRSC	Box	Sofamor Danek	0.30	0.20	0.20
7	Syncage C	Box	Synthes	0.26	0.26	0.21
8	Harms	Cylinder	DePuyAcroMed	0.10	0.10	0.10
9	MSD	Cylinder	Ulrich	0.20	0.32	0.32
10	Kaden	Cylinder	BiometMerck	0.84	1.21	1.21

* The producer recommends the use of two parallel inserted cages of this height for cervical interbody fusion.
EICA = endplate implant contact area; u = upper; l = lower.

ment stiffness of the Syncage was significantly greater in all directions ($P < 0.001$).

Cylinder Designs. In comparison with the intact motion segment, the flexion, extension, and rotation stiffness of all the cages with the cylinder design was significantly greater ($P < 0.05$). Rotation stiffness of the cages increased with the endplate–implant contact area. Therefore, in comparison with the intact motion segment, the rotation stiffness of the Harms and MSD cages was significantly less ($P < 0.05$), whereas that of the Kaden cage was significantly greater ($P < 0.01$).

Comparison Between Cages and Tricortical Bone Graft

Screw Designs. In comparison a with bone graft, all the cages with screw designs were less stable during flexion, extension, and bending ($P < 0.01$). No significant difference was found in rotation stiffness between the three cage types with a screw design and bone graft.

Box Designs. In comparison with bone graft, the flexion stiffness of the Novus CSRC was significantly less ($P < 0.01$). No significant difference was found in extension and bending stiffness between the Novus CSRC and bone graft fixated motion segment. The rotation stiffness was greater in the Novus CSRC ($P < 0.05$). In comparison with bone graft, the stiffness of the Syncage was significantly greater in all directions ($P < 0.001$).

Cylinder Designs. In comparison with bone graft, the flexion stiffness of all the cages with a cylinder design was significantly lower ($P < 0.05$), whereas the extension stiffness was not different. The bending stiffness was significantly greater ($P < 0.05$). In comparison with bone graft, the rotation stiffness was greater for the Kaden cage ($P < 0.05$), not different for the MSD cage, and significantly lower for the Harms cage ($P < 0.05$).

Intragroup Comparison

Screw Design. No significant difference was found between the three cage types with screw design except for bending stiffness of the BAK 12 mm, which was significantly lower than the bending stiffness of the other two cage types ($P < 0.01$).

Box Design. The stiffness of the Syncage was significantly greater than the stiffness of the Novus CSRC in all directions ($P < 0.01$).

Cylinder Design. No difference was found between the three cage types with a cylinder design for flexion, extension, and bending stiffness. The rotation stiffness of the cylindrical cages increased with the endplate–implant contact area. Therefore, the rotation stiffness of the Kaden cage was significantly greater than that of the Harms ($P < 0.001$) and the MSD cage ($P < 0.01$).

Intergroup Comparison

Screw Design *Versus* Box Design. The stiffness of all the screw designs was significantly less than the stiffness of the Syncage in all directions ($P < 0.01$). If two screws were inserted (Novus CTTi and BAK-C 8 mm), the flexion stiffness for the screw designs was higher than for the Novus CSRC ($P < 0.05$). In contrast, no difference was observed if one screw was inserted (BAK-C 12 mm). No significant difference was found between the Novus CTTi and the Novus CSRC in extension, rotation, and bending. Extension, rotation, and bending stiffness was significantly higher for the Novus CSRC than for both BAK-C cages ($P < 0.05$).

Screw Design *Versus* Cylinder Design. If two screws were inserted (Novus CTTi and BAK-C 8 mm), no significant difference was observed in flexion stiffness between the two design groups. If one screw was inserted (BAK-C 12 mm), flexion stiffness was higher for the cylinder designs ($P < 0.05$). Extension and bending stiffness were always higher with the cylinder designs ($P < 0.05$). If two screws were inserted (Novus CTTi and BAK-C 8 mm), the rotation stiffness was greater than with the Harms cage ($P < 0.01$), not different than with the MSD cage, and less than with the Kaden cage ($P < 0.01$). If one screw was inserted (BAK-C 12 mm), the rotation stiffness did not differ from that of the Harms cage, but was less than that of the MSD cage ($P < 0.05$) and the Kaden cage ($P < 0.01$).

Box Design *Versus* Cylinder Design. The stiffness of all the cylinder designs was significantly less than that of the

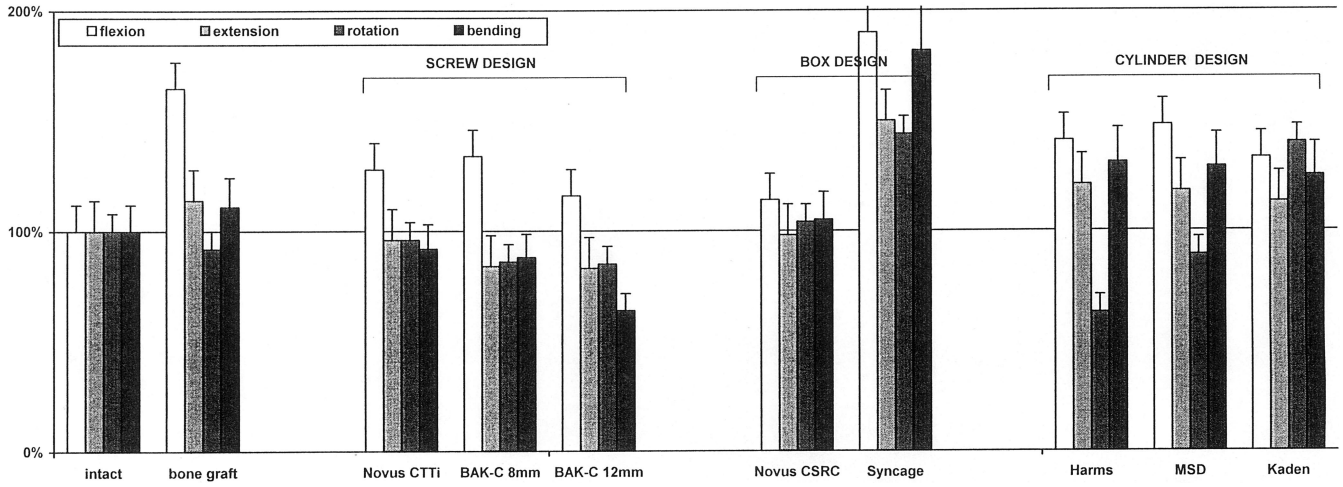


Figure 2. The stiffness of different cervical spine interbody fusion cages normalized to the intact motion segment C3–C4 in response to flexion, extension, rotation, and bending.

Syncage in all directions ($P < 0.01$). Flexion, extension, and bending stiffness was greater than for the cages with the cylinder design than for the Novus CSRC. The rotation stiffness of the Novus CSRC was greater than that of the Harms cage ($P < 0.001$) and the MSD cage ($P < 0.05$), but lower than that of the Kaden cage ($P < 0.01$).

Volume-Related Stiffness

Figure 3 summarizes the volume-related stiffness results for the cervical spine interbody fusion cages normalized with respect to the bone graft during flexion, extension, rotation, and bending.

In comparison with bone graft, all the cages increased significantly in volume-related stiffness. The volume-related stiffness for flexion extension and bending was significantly greatest for the Harms cage ($P < 0.01$). There was no difference for rotation volume-related stiffness between the Harms cage and the Syncage.

■ Discussion

Study Limitations

A variety of different test loading conditions have been used in the past, making comparison of the results from different groups almost impossible. Therefore, according to previously published recommendations,[47] the *in vitro* tests in this study were performed under pure moment loading conditions. Because of the complex and still widely unknown loading of the cervical spine *in vivo,* it is impossible to transfer the results of this sheep *in vitro* study directly to the human cervical spine *in vivo*. Therefore, the biomechanical performance of the tested implants in the human *in vivo* may differ significantly from the results obtained in this sheep *in vitro* study. Additionally, because of the complex loading conditions of the human cervical spine *in vivo,* it is not possible to determine the correlation between the 25-N compression load

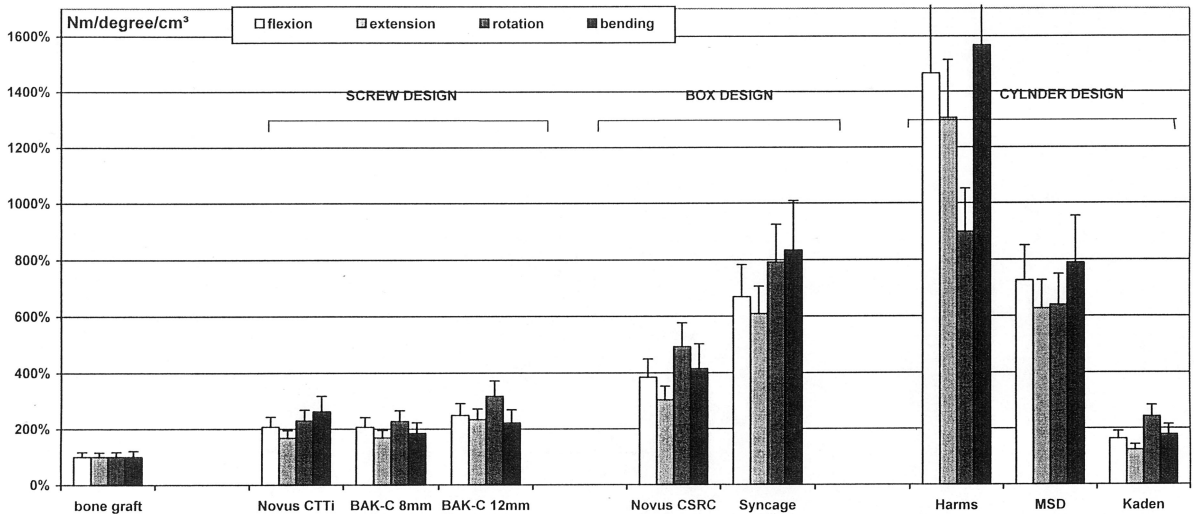


Figure 3. The volume-related stiffness of different cervical spine interbody fusion cages normalized to the bone graft in response to flexion, extension, rotation, and bending.

and the 6-Nm moment in this *in vitro* sheep experiment with *in vivo* human loading conditions. However, new implants should be tested *in vitro* for primary stability in standardized laboratory tests to determine which implant is the most appropriate before its acceptance for clinical use.

Animal Model

In spine research, the sheep spine frequently is used as a model for the human spine.[1,5,7,24,25,27,29,38,45,46,49] Recently, anatomic biomechanical, and bone mineral density evaluation of the sheep cervical spine has shown good comparability with the human spine.[25,45,46] Anatomic evaluation has demonstrated an average disc space height of 6 mm in the sheep cervical spine, which is approximately 1 mm higher than in the human cervical spine.[30] Additionally, similar upper endplate parameters of C4 and C5 in both species were observed. Therefore, human implants fit in the sheep cervical spine.

Wilke et al[45] were the first to describe biomechanical analogies between sheep and human spines by testing sheep spines and comparing the data to the literature. Although many data are available from *in vitro* and *in vivo* tests of the normal human cervical spine,[31,42,44] comparison remains difficult because of the widely varied test setups. Kandziora et al[15] compared both species biomechanically using the same test setup. Although significant differences were observed between the two species, especially for rotation parameters, range of motion and stiffness data were mainly comparable in their craniocaudal trends. These researchers concluded that if the sheep animal model is used, motion segments C2–C3 and C3–C4 were the most suitable for biomechanical tests.

Bone mineral density has an influence on the primary stability of cervical spine fixation techniques.[8,14,18,20,36,48] In particular, a linear correlation was observed between the axial compression strength of cages and bone mineral density.[12] Currently, no data are available comparing the bone mineral density of severely degenerated human cervical spine motion segments for which surgery is indicated with that of the sheep cervical spine. However, in a study comparing bone mineral density values between human and sheep cervical spines,[15] no significant difference could be registered with regard to bone mineral density between the two species. The least percentile difference was found for C4. Standard deviations for bone mineral density of the human cervical spine were four times greater than for that of the sheep cervical spine. Therefore, the sheep animal model, in particular the motion segment C3–C4, seems to allow a quasi-"bone mineral density–independent" biomechanical evaluation of human size implants.

Stiffness Tests

Primary stability in cervical spine interbody fusion is determined essentially by the biomechanical effects of the fixation devices.

Several biomechanical studies were performed to evaluate lumbar interbody fusion cages with screw designs.[2,9,10,12,16,21,28,32–34,40,41,50] The main interest of these studies was to compare different screw designs or to evaluate the biomechanical effects of additional posterior fixation techniques. Zdeblick et al[50] and Rapoff et al,[34] using two BAK devices in calf spines, found that bending and flexion–extension stiffness increased twofold as compared with noninstrumented motion segments. Comparable results were obtained by Nibu et al,[28] Rapoff et al,[34] and Rathonyi et al[35] in studies of the human spine. Tencer et al[40] and Kettler et al[16] made the same observation using the Ray device, another interbody fusion cage with a screw design. In contrast, Pitzen et al[32,33] using posterior lumbar interbody fusion techniques with the BAK cage in an *in vitro* biomechanical and finite element model showed a loss of stiffness in compression, shearing, and especially torsion. Interestingly, all investigators noted that as compared with the intact spine, torsional stiffness was minimally changed or decreased by the use of screwlike implants.

Very little information on biomechanical testing of cages with cylinder designs is available. Heller et al[9] used the Harms titanium cage in a calf lumbar spine corporectomy model, showing that only an anteroposterior construct was able to restore stability comparable with that of the intact state. To the authors' knowledge, Hollowell et al[10] were the first to use the Harms titanium cage in a biomechanical study applying a discectomy model of the human thoracic spine. These researchers focused mainly on subsidence without testing the three-dimensional displacement stiffness of this implant. Pitzen et al[32,33] used the Harms titanium cage in a lumbar spine discectomy model. Their results showed a greater initial stability for posterior lumbar interbody fusion with the Harms titanium cage and posterior instrumentation than for posterior lumbar interbody fusion with the BAK cage alone.

As with cylinder designs, information about biomechanical data of cages with box designs is scarce. Brantigan et al,[2] using a posterior lumbar interbody fusion model in the lumbar spine, demonstrated that their carbon fiber implant had superior biomechanical properties, as compared with those of the bone graft. To the authors' knowledge, only Shono et al[37] performed biomechanical studies in the cervical spine, demonstrating that the Brantigan carbon fiber cage[2] was more rigid than an iliac bone graft.

The aim of the current study was to evaluate the biomechanical effects of cervical spine interbody fusion cages, and to compare three CSIFC design groups. To the authors' knowledge, no biomechanical study addressing different cervical spine interbody fusion cages is currently available. Additionally, no data are available that compare different cage design groups. Cage size has a significant influence on the biomechanical behavior of different cages.[6] Therefore, to allow comparison be-

tween the different cage designs, cages of similar height, width, and depth were used in this study.

Any cage implantation increased flexion stiffness over that of the intact motion segment. In contrast, rotation stiffness decreased after implantation of a CSIFC, except for the Novus CSRC, Syncage, and Kaden cage. As compared with the intact motion segment, extension and bending stiffness increased with the cylinder designs and with the Syncage, a box design, whereas extension and bending stiffness for screw designs decreased or remained unchanged.

Comparison between the different cage designs showed that if two screws were inserted (Novus CTTi and BAK-C 8 mm), no significant difference in flexion stiffness between screw and cylinder design groups was registered. Yet, if one screw was inserted (BAK-C 12 mm), flexion stiffness was greater for cylinder designs ($P < 0.05$). Extension and bending stiffness were always greater with cylinder designs ($P < 0.05$). The best biomechanical results were observed in the Syncage, a box design.

Volume-Related Stiffness

Secondary stability in cervical spine interbody fusion is determined biologically, resulting from the amount of bony fusion.

The ideal biologic environment for spondylodesis is influenced by several factors including optimal grafting techniques, maximum graft filling of the intervertebral space, mechanical protection of the graft in the intervertebral space, and optimal surface contact area of graft and vertebral body.[43] However, as cage volume increases, graft volume decreases. Therefore, some kind of competition exists between cage and graft volume. In conclusion, one important biologic factor for a fusion cage is having the smallest possible cage volume, which allows the maximum graft filling of the intervertebral space.[43] However, cages also must provide adequate mechanical stability. In an attempt to take these two factors into consideration during the current study, the volume-related stiffness was determined by dividing stiffness results by cage volume. This parameter should allow the interpretation of biomechanical test results from a more biologic point of view.

The volume-related stiffness was significantly highest with the Harms cage and the Syncage. In conclusion, with these cages, less implant in needed for mechanical stabilization. Therefore, sufficient space remains to be filled by bone graft, which in turn might lead to a better biologic environment for bony fusion. Several studies[17,39] have documented that if graft volume increases, the chance for bony fusion also increases. Therefore, the data suggest that the Harms cage and the Syncage probably will provide a better biologic environment for spondylodesis than the other cages tested. Further *in vivo* studies with different cage designs are necessary to determine whether the volume-related stiffness parameter is able to predict secondary stability in cervical spine interbody fusion.

■ Conclusion

The ideal CSIFC should correct an existing deformity, provide stability to the segment until fusion occurs, and supply a good environment for successful spondylodesis without concurrent morbidity associated with its use.[43] This study aimed to evaluate the mechanical properties of CSIFC and to compare three CSIFC design groups. From biomechanical perspectives, the results suggest that intragroup design variations in the screw and cylinder design groups are of little importance. However, cages with a cylinder design were able to control extension and bending more effectively than cages with a screw design.

A question that cannot be answered by this *in vitro* study concerns the level of rigidity required to obtain long-term stability and fusion by cervical spine interbody fixation methods. It may be assumed, however, that the more spinal motion is eliminated, the greater will be the chance of definite spinal fusion. Therefore, it seems reasonable that the most reliable and rigid fixation method would be the method of choice. In conclusion, the authors suggest the use of cages with a cylinder or box design for cervical spine interbody fusion.

Another unanswered question concerns the secondary stability of CSIFC. Volume-related stiffness may allow the interpretation of mechanical test results from a more biologic point of view, but further *in vivo* investigations are necessary.

■ Key Points

- Biomechanical results indicate that design variations in screw and cylinder design groups are of little importance.
- Cages with cylinder design were able to control extension and bending more effectively than cages with screw design.
- Volume-related stiffness might allow interpretation of biomechanical test results from a more biological point of view.

References

1. Ahlgren BD, Vasavada MS, Brower RS, et al. Annular incision technique on the strength and multidirectional flexibility of the healing intervertebral disc. Spine 1994;19:948–54.
2. Brantigan JW, Steffee AD, Geiger JM. A carbon fiber implant to aid interbody lumbar fusion: mechanical testing. Spine 1991;16:277–82.
3. Brooke NS, Rorke AW, King AT, et al. Preliminary experience of carbon fibre cage prostheses for treatment of cervical spine disorders. Br J Neurosurg 1997;11:221–7.
4. Brodke DS, Dick JC, Kunz DN, et al. Posterior lumbar interbody fusion: a biomechanical comparison, including a new threaded cage. Spine 1997;22:26–31.
5. Cain CC, Fraser RD. Bony and vascular anatomy of the normal cervical spine in the sheep. Spine 1995;20:759–65.
6. Goh JC, Wong HK, Thambyah A, et al. Influence of PLIF cage size on lumbar spine stability. Spine 2000;25:35–9.

7. Gunzburg R, Fraser RD, Moore R, et al. An experimental study comparing percutaneous discectomy with chemonucleolysis. Spine 1993;18:218–26.
8. Halvorson TL, Kelley LA, Thomas HA, et al. Effects on bone mineral density on pedicle screw fixation. Spine 1994;19:2415–20.
9. Heller JG, Zdeblick TA, Kunz DA, et al. Spinal instrumentation for metastatic disease: *in vitro* biomechanical analysis. J Spinal Disord 1993;6:17–22.
10. Hollowell JP, Vollmer DG, Wilson CR, et al. Biomechanical analysis of thoracolumbar interbody constructs: how important is the endplate? Spine 1996;21:1032–6.
11. Jensen J, Kragskov J, Wenzel A, et al. Volumetry of bone grafts by three-dimensional computed tomographic reconstruction: an animal study in the minipig. Dentomaxillofac Radiol 1998;27:41–4.
12. Jost B, Cripton PA, Lund T, et al. Compressive strength of interbody cages in the lumbar spine: the effect of cage shape, posterior instrumentation, and bone density. Eur Spine J 1998;7:132–41.
13. Kandziora F, Kerschbaumer F, Starker M, et al. Biomechanical assessment of the transoral plate fixation for atlantoaxial instability. Spine 2000;25:1555–61.
14. Kandziora F, Mittlmeier T, Kerschbaumer F. Stage-related surgery for cervical spine instability in rheumatoid arthritis. Eur Spine J 1999;8:371–81.
15. Kandziora F, Pflugmacher R, Scholz M, et al. Comparison between sheep and human cervical spines: an anatomic, radiographic, bone mineral density, and biomechanical study. Spine 2001;26:1028–37.
16. Kettler A, Wilke HJ, Dietl R, et al. Stabilising effect of posterior lumbar interbody fusion cages before and after cyclic loading. J Neurosurg 2000;92:87–92.
17. Kim KW, Ha KY, Moon MS, et al. Volumetric change of the graft bone after intertransverse fusion. Spine 1999;24:428–33.
18. Kumano K, Hirabayashi S, Ogawa Y, et al. Pedicle screws and bone mineral density. Spine 1994;19:1157–61.
19. Kumaresan S, Yoganandan N, Pintar FA. Finite element analysis of anterior cervical spine interbody fusion. Biomed Mater Eng 1997;7:221–30.
20. Lim TH, An HS, Evanich C, et al. Strength of vertebral screw fixation in relationship to bone mineral density. J Spinal Disord 1995;8:121–5.
21. Lund T, Oxland TR, Jost B, et al. Interbody cage stabilisation in the lumbar spine: biomechanical evaluation of cage design, posterior instrumentation, and bone density. J Bone Joint Surg [Br] 1998;80:351–9.
22. Majd ME, Vadhva M, Holt RT. Anterior cervical reconstruction using titanium cages with anterior plating. Spine 1999;24:1604–10.
23. Matge G. Anterior interbody fusion with the BAK-cage in cervical spondylosis. Acta Neurochir 1998;140:1–8.
24. Melrose J, Ghosh P, Taylor TKF, et al. A longitudinal study of the matrix changes induced in the intervertebral discs by surgical damage to the annulus fibrosus. J Orthop Res 1992;10:665–76.
25. Moore RJ, Osti OL, Vernon-Roberts B, et al. Changes in endplate vascularity after an outer annulus tear in the sheep. Spine 1992;17:874–8.
26. Munoz FLO, de las Heras BG, Lopez VC, et al. Comparison of three techniques of anterior fusion in single-level cervical disc herniation. Eur Spine J 1998;7:512–6.
27. Nagel DA, Kramers PC, Rahn BA, et al. A paradigm of delayed union and nonunion in the lumbosacral joint: a study of motion and bone grafting of the lumbosacral spine in sheep. Spine 1991;16:553–9.
28. Nibu K, Panjabi MM, Oxland T, et al. Multidirectional stabilising potential of BAK interbody spinal fusion system for anterior surgery. J Spinal Disord 1997;10:357–62.
29. Osti OL, Vernon-Roberts B, Fraser RD. Annulus tear in intervertebral disc degeneration: an experimental study using an animal model. Spine 1990;15:431–5.
30. Pait GT, Killefer JA, Arnautovic KI. Surgical anatomy of the anterior cervical spine: the disc space, vertebral artery, and associated bony structures. Neurosurgery 1996;39:769–76.
31. Panjabi MM, Duranceau J, Goel V, et al. Cervical human vertebrae: quantitative three-dimensional anatomy of the middle and lower regions. Spine 1991;16:861–9.
32. Pitzen T, Caspar W, Matthis D, et al. Primary stability of two PLIF (posterior lumbar interbody fusion): a biomechanical *in vitro* comparison. Z Orthop 1999;137:214–8.
33. Pitzen T, Matthis D, Caspar W, et al. Initial stability of two PLIF-techniques: a biomechanical comparison using a finite element model. Orthopäde 2000;29:68–72.
34. Rapoff AJ, Ghanayem AJ, Zdeblick TA. Biomechanical comparison of posterior lumbar interbody fusion cages. Spine 1997;22:2375–9.
35. Rathonyi GC, Oxland TR, Gerich U, et al. The role of supplemental translaminar screws in anterior interbody fixation: a biomechanical study. Eur Spine J 1998;7:400–7.
36. Ryken TC, Goel VK, Clausen JD, et al. Assessment of unicortical and bicortical fixation in a quasistatic cadaveric model: role of bone mineral density and screw torque. Spine 1995;20:1861–7.
37. Shono Y, McAfee PC, Cunningham BW, et al. A biomechanical analysis of decompression and reconstruction methods in the cervical spine: emphasis on a carbon fiber composite cage. J Bone Joint Surg [Am] 1993;75:1674–84.
38. Slater R, Nagel R, Smith RL. Biochemistry of fusion mass consolidation in the sheep spine. J Orthop Res 1988;6:138–44.
39. Stevenson S, Emery SE, Goldberg VM. Factors affecting bone graft incorporation. Clin Orthop 1996;324:66–74.
40. Tencer AF, Hampton D, Eddy S. Biomechanical properties of threaded inserts for lumbar interbody spinal fusion. Spine 1995;20:2408–14.
41. Volkman T, Horton WC, Hutton WC. Transfacet screws with lumbar interbody reconstruction: biomechanical study of motion segment stiffness. J Spinal Disord 1996;9:425–32.
42. Voo LM, Pintar FA, Yoganandan N, et al. Static and dynamic bending response of the human cervical spine. J Biomech Eng 1998;120:693–6.
43. Weiner BK, Fraser RD. Spine update lumbar interbody fusion cages. Spine 1998;23:634–40.
44. Wen N, Lavaste F, Santin JJ, et al. Three-dimensional biomechanical properties of the human spine *in vitro*. Eur Spine J 1993;2:2–47.
45. Wilke HJ, Kettler A, Claes LE. Are sheep spines a valid biomechanical model for human spines? Spine 1997;22:2365–74.
46. Wilke HJ, Kettler A, Wenger KH, et al. Anatomy of the sheep spine and its comparison to the human spine. Anat Rec 1997;247:542–55.
47. Wilke HJ, Wenger K, Cleas L. Testing criteria for spinal implants: recommendations for the standardization of *in vitro* stability of spinal implants. Eur Spine J 1998;7:148–54.
48. Wittenberg RH, Shea M, Swartz DE, et al. Importance of bone mineral density in instrumented spine fusions. Spine 1991;16:647–52.
49. Yamamuro T, Shikata J, Okumura H, et al. Replacement of the lumbar vertebrae of sheep with ceramic prostheses. J Bone Joint Surg [Br] 1990;72:889–93.
50. Zdeblick TA, Warden KE, Zou D, et al. Anterior spinal fixators: a biomechanical *in vitro* study. Spine 1993;18:513–7.

Address reprint requests to

Frank Kandziora, MD
Unfall- und Wiederherstellungschirurgie
Universitätsklinikum Charité der Humboldt
Universität Berlin
Campus Virchow Klinikum
Augustenburgerplatz 1
13353 Berlin
Germany
E-mail: frank.kandziora@charite.de

Diese Untersuchung konnte zeigen, dass Cages mit Schraubendesign nur eine geringe Primärstabilität in vitro aufweisen. Hingegen legt die in dieser Untersuchung dokumentierte hohe in vitro Primärstabilität des Syncage-C dessen in vivo Evaluation nahe. Der aus dieser Untersuchung hervorgegangene Parameter der Volumen-bezogenen-Steifigkeit macht darüber hinaus auch die Evaluation des Harmscages in vivo interessant, obwohl dessen in vitro Primärstabilität deutlich geringer ist als die des Syncage-C.

2.2.2 In vivo Untersuchung zervikaler Cages

In klinischen Untersuchungen finden sich Hinweise, dass Cages im Vergleich zum trikortikalen Beckenkammspan in der Lage sind, die Ergebnisse der intervertebralen Spondylodese zu verbessern [19,21,66,67,111,112,113,152]. Zusätzlich konnte trotz ähnlicher Evaluationsprotokolle eine relevante Variabilität des operativen Erfolges bei Verwendung verschiedener zervikaler Cagedesigns demonstriert werden [66,67,152,155]. Ob diese Ergebnisvariabilität eine Folge von operativen Faktoren ist oder ob das Implantatdesign der Cages hierbei eine entscheidende Rolle spielt, ist derzeit unbekannt.

- Daher sollten in dieser Untersuchung in einem operativ standardisiertem Tiermodell unterschiedliche Cagedesigns hinsichtlich radiologischer, biomechanischer und histologischer Parameter der Einheilung miteinander verglichen werden.
- Zusätzlich sollte evaluiert werden, ob Spongiosa gefüllte Cages der herkömmlichen Spondylodese mit autologem trikortikalem Beckenkammspan hinsichtlich radiologischer, biomechanischer und histologischer Parameter während der Einheilung gleichwertig sind.

J Neurosurg (Spine 3) 96:321–332, 2002

Influence of cage design on interbody fusion in a sheep cervical spine model

FRANK KANDZIORA, M.D., GEORG SCHOLLMEIER, M.D., MATTI SCHOLZ, JAN SCHAEFER, ALEXANDRA SCHOLZ, GERHARD SCHMIDMAIER, M.D., RALF SCHRÖDER, M.D., HERMAN BAIL, M.D., GEORG DUDA, PH.D., THOMAS MITTLMEIER, M.D., AND NORBERT P. HAAS, M.D.

Unfall- und Wiederherstellungschirurgie, Universitätsklinikum Charité der Humboldt-Universität Berlin, Campus Virchow-Klinikum, Berlin; and Strahlenklinik und Poliklinik, Universitätsklinikum Charité der Humboldt-Universität Berlin, Campus Virchow-Klinikum, Berlin, Germany

Object. The purpose of this study was to compare the characteristics of interbody fusion achieved using an autologous tricortical iliac crest bone graft with those of a cylinder- and a box-design cage in a sheep cervical spine model. This study was designed to determine whether there are differences between three interbody fusion procedures in: 1) ability to preserve postoperative distraction; 2) biomechanical stability; and 3) histological characteristics of intervertebral bone matrix formation.

Methods. Twenty-four sheep underwent C3–4 discectomy and fusion in which the following were used: Group 1, autologous tricortical iliac crest bone graft (eight sheep); Group 2, titanium cylinder-design cage filled with autologous iliac crest bone graft (eight sheep); and Group 3, titanium box-design cage filled with autologous iliac crest graft (eight sheep). Radiography was performed pre- and postoperatively and after 1, 2, 4, 8, and 12 weeks. At the same time points, disc space height, intervertebral angle, and lordosis angle were measured. After 12 weeks, the sheep were killed, and fusion sites were evaluated by obtaining functional radiographs in flexion and extension. Quantitative computerized tomography scans were acquired to assess bone mineral density, bone mineral content, and bone callus volume. Biomechanical testing was performed in flexion, extension, axial rotation, and lateral bending. Stiffness, range of motion, neutral zone, and elastic zone were determined. Histomorphological and histomorphometric analyses were performed, and polychrome sequential labeling was used to determine the time frame of new bone formation.

Over a 12-week period significantly higher values for disc space height and intervertebral angle were shown in cage-treated sheep than in those that received bone graft. Functional radiographic assessment revealed significantly lower residual flexion–extension movement in sheep with the cylinder cage–fixed spines than in those that received bone graft group. The cylinder-design cages showed significantly higher values for bone mineral content, bone callus content, and stiffness in axial rotation and lateral bending than the other cages or grafts. Histomorphometric evaluation and polychrome sequential labeling showed a more progressed bone matrix formation in the cylindrical cage group than in both other groups.

Conclusions. Compared with the tricortical bone graft, both cages showed significantly better distractive properties. The cylindrical cage demonstrated a significantly higher biomechanical stiffness and an accelerated interbody fusion compared with the box-design cage and the tricortical bone graft. The differences in bone matrix formation within both cages were the result of the significantly lower stress shielding on the bone graft by the cylinder-design cage.

KEY WORDS • cervical spine • interbody cage • spinal fusion • sheep

ANTERIOR decompression and interbody fusion are a widely accepted surgical treatment for patients with cervical spondylosis. Until now, tricortical iliac crest bone graft has been the gold standard, although it is associated with a high rate of donor-site morbidity. Additional problems such as pseudarthrosis, graft collapse–induced kyphotic deformity, and graft extrusion have led to a rapid increase in the use of cervical spine interbody fusion cages as an adjunct to arthrodesis,[4,17,21,24,25,27] although experimental data are lacking.[37]

Several interbody construct designs have been developed. According to Weiner and Fraser[37] cage designs can be subdivided into three groups: screw (horizontal cylinder), box, or cylinder (vertical ring) designs.

Devices used for interbody stabilization must ensure good primary stability. Cages have been specially designed to provide immediate strong anterior column support. This effect has already been proven in several bio-

Abbreviations used in this paper: BCV = bone callus volume; BMC = bone mineral content; BMD = bone mineral density; CT = computerized tomography; DSH = disc space height; EZ = elastic zone; IVA = intervertebral angle; LA = lordotic angle; NZ = neutral zone; ROI = region of interest; ROM = range of motion; SD = standard deviation; 2D = two-dimensional.

mechanical in vitro studies in which all cage designs were evaluated.[2,3,11–14,19,21,23,28,31–33,35,36] In particular, in vitro biomechanical fixation properties of screw-design cages have been evaluated extensively.[3,11,14,19,23,28,31,32,35] Only scarce data, however, are available concerning comparative evaluation of different cage designs. Recently, Oxland, et al.,[29] compared a box- and a cylinder-design cage in a biomechanical clinical in vitro study and found no differences between the two cages. In contrast, Mittlmeier, et al.,[26] found fundamental differences among box-, screw- and cylinder-design cages in a sheep cervical spine model. They demonstrated that cylinder-design and box-design cages were able to control extension and bending more effectively than screw-design cages.

Additionally, interbody cages have been developed to provide interspace structural stability during bone fusion. Cages should retain interbody distraction and should be resistant to subsidence into the adjacent vertebrae to foster a biological environment that guarantees the desired quality of osseous fusion. This can only be evaluated, however, by performing in vivo investigations. The structural stability of screw-design cages has already been proven by authors of some in vivo studies,[34,38] who have shown that these cages preserved interbody distraction more effectively than autologous tricortical iliac crest bone graft. Experimental in vivo data for cylinder- or box-design cages, however, are not available. Additionally, no information is available concerning comparative experimental in vivo evaluation of different cage designs.

Therefore, the purpose of this study was to compare a developing interbody fusion fostered by an autologous tricortical iliac crest bone graft with those of a cylinder- and a box-design cage in a sheep cervical spine interbody fusion model. This study was designed to determine whether there were differences, at a given early time point, in a developing fusion mass between the three interbody fusion techniques with regard to: 1) ability to preserve postoperative distraction; 2) biomechanical stability; and 3) histological characteristics of intervertebral bone matrix formation.

Materials and Methods

Study Design

Twenty-five (2-year-old) adult female merino sheep underwent C3–4 discectomy and fusion; one animal was lost to follow up. The remaining 24 sheep were randomly assigned to the following groups: Group 1, autologous tricortical iliac crest graft (eight sheep); Group 2, cylinder-design titanium cage filled with autologous cancellous iliac crest bone graft (eight sheep); and Group 3, box-design titanium cage filled with autologous cancellous iliac crest graft (eight sheep).

The sheep were evaluated prospectively for 12 weeks, after which they were killed and underwent radiographic, biomechanical, and histological evaluations. All animal-related experimental work was approved by local authorities.

Surgical Technique and Postoperative Care

All sheep received 2 g amoxicillin intravenously before surgery. Surgery was performed after induction of general endotracheal anesthesia (0.5 g thiopental-natrium and 0.1 mg fentanyl citrate). For maintenance of anesthesia, inhalational isofluran and intravenous dosages of 0.2 mg fentanyldihydrogencitrat were applied. The anterior part of the neck and the left iliac crest was prepared in a sterile fashion, and a left anterolateral approach to the cervical spine was undertaken through a longitudinal skin incision. The longus colli muscle was incised in the midline, and the intervertebral C3–4 disc was exposed. After a Caspar device was used to distract the motion segments, anterior C3–4 discectomy was performed. The endplates were shaved using a 2-mm high-speed diamond drill down to bleeding bone, resulting in an excision of 1 mm of each endplate. No attempt was made to excise the posterior longitudinal ligament or expose the spinal canal. For interbody stabilization, titanium cylinder-design cages (Group 2, Harms cage; width 14 mm, depth 14 mm, and cage volume 0.1 cm^3) or box-design cages (Group 3, SynCage-C; width 15 mm, depth 13 mm, and cage volume 0.26 cm^3) of appropriate height (mean height 8 mm for both groups) were used. Prior to insertion, cages were filled with autologous cancellous bone grafts. In Group 1 a tricortical autologous bone graft (mean height 8 mm, mean depth 14 mm, and 11 mm average width) was taken from the left iliac crest. Prior to insertion the volumes of the cages filled with autologous bone or the volumes of the bone grafts were determined using water displacement technique (Archimedes principle). The tricortical bone graft was inserted press-fit into the intervertebral space with the cortical shape of the graft anterior (Robinson technique). Finally, the wound was irrigated with saline, and the longus colli muscle was closed using a running suture. The subcutaneous tissue and skin were reapproximated using interrupted sutures, and a soft bandage was applied to the neck.

After surgery, the sheep were observed until fully recovered from anesthesia. They received two 0.5-g doses of metamizol-natrium per day for 5 days intramuscularly. Clinical examination was performed daily for the first 10 days and weekly thereafter. The sheep were allowed ad libitum activity for the remainder of the experiment. Fluorochrome sequential labeling was performed at 3, 6, and 9 weeks postoperatively (oxytetracyclin [25 mg/kg intravenously] at 3 weeks, calcein green [15 mg/kg intravenously] at 6 weeks, and xylenol orange [90 mg/kg intravenously] at 9 weeks). Twelve weeks after surgery, after induction of anesthesia (0.5 g thiopental-natrium and 0.1 mg fentanyldihydrogencitrat) the sheep were killed by an intravenous injection of potassium chloride. The complete cervical spine, including parts of the occiput and T-1, was excised and cleaned from the surrounding tissue.

Radiographic Evaluation

To allow for comparable radiographic evaluation, special fixation devices for the sheep cervical spine were developed. The reproducibility of the positioning of the sheep cervical spine in this fixation device was investigated by repeated measurements. Prior to surgery, one sheep in each group was selected randomly to undergo radiography, and 10 lateral and 10 anteroposterior digital radiographs were obtained. After each radiograph was acquired, the sheep was removed from the fixation device, turned around, and then repositioned in the fixation device. Thus, the complete series of linear and angular measurements was performed on each radiograph.

Lateral and anteroposterior digital radiographs were acquired pre- and postoperatively and after 1, 2, 4, 8, and 12 weeks. During the same time periods anterior, middle, and posterior intervertebral DSH, IVA, and LA of the C3–4 motion segment were measured on lateral radiographs (Fig. 1). The mean intervertebral DSH was calculated from anterior, middle and posterior DSH measurements (anterior + middle + posterior DSH/3). After 12 weeks, bone fusion was categorized using the following parameters: A) no bone fusion; B) maximum intervertebral gap of more than 5 mm; C) maximum intervertebral gap of less than 5 mm; and D) complete bone fusion. The maximum intervertebral gap in the craniocaudal direction was measured directly on lateral x-ray films by using a ruler. All radiographic measurements were evaluated by three independent observers.

Functional Radiographic Analysis

After sacrifice fusion sites were evaluated using lateral digital functional radiographic scans in flexion and extension (Fig. 2). For this purpose, T-1 was rigidly fixed with a Steinmann pin while a 60-N load was applied through C-1 by using a newton meter. Flex-

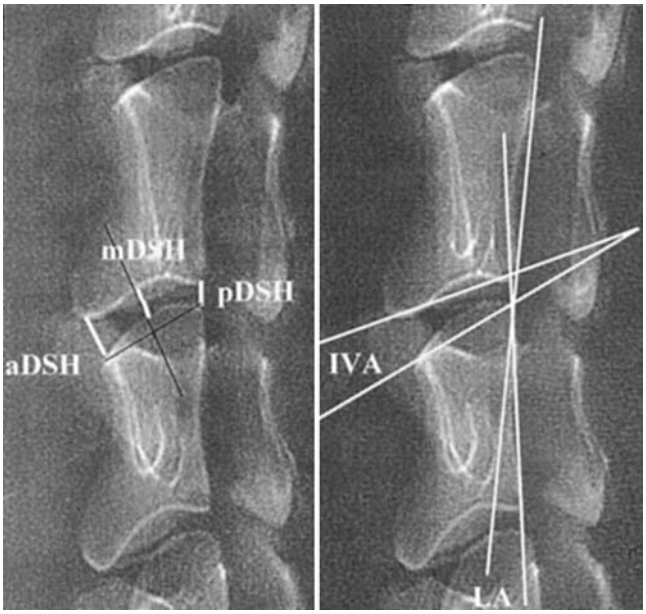


FIG. 1. Radiographic measurements. *Left:* Anterior DSH (aDSH), posterior DSH (pDSH), and middle DSH (mDSH). *Right:* The IVA and LA.

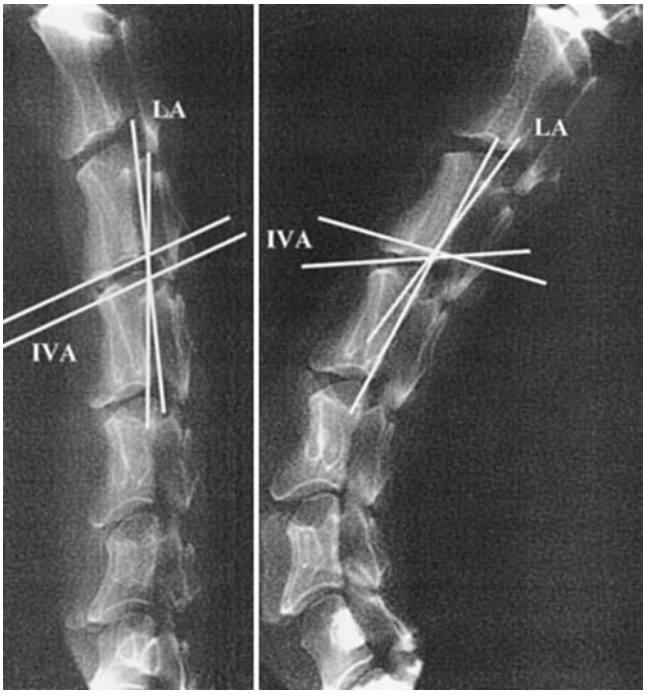


FIG. 2. The C3–4 motion segment was evaluated using lateral digital functional radiography in flexion *(left)* and extension *(right)*. Flexion–extension differences of IVA and LA were calculated.

ion–extension differences in IVA and LA were calculated. All functional radiographic measurements were evaluated by three independent observers.

Quantitative CT Analysis

After the sheep were killed, quantitative CT scanning was performed using a scanner. Axial cuts with 1-mm slice thickness were made parallel to the intervertebral disc space. Bone mineral density measurements of the bone callus have been described in detail earlier.[18] Measurements were calibrated with a six-point BMD phantom and were performed using software designed specifically for the scanner. Bone callus volume was measured using an image analyzing system. Bone mineral content was calculated from BMD and BCV measurements (BMC = BCV × BMD). After 12 weeks bone fusion was categorized on sagittal and coronal 2D CT reconstructions by using the aforementioned parameters. The maximum intervertebral gap in the craniocaudal direction was measured directly on midsagittal 2D CT reconstructions by using the specifically designed scanner software. All CT measurements were evaluated by three independent observers.

Biomechanical Analysis

After the sheep were killed, the specimens were biomechanically tested in a nonconstrained testing apparatus by using a nondestructive flexibility method previously described.[16,18] Pure bending moments were applied to the C3–4 motion segments by using a system of cables and pulleys to induce flexion, extension, left and right lateral bending, and left and right axial rotation. Tension was applied to the cables with a uniaxial testing machine. Three-dimensional displacement of each motion segment was measured using an optical measurement system. Triangular markers with three diodes were attached to the vertebral bodies of C-3 and C-4. Marker positions were detected using two cameras and recorded using a computerized motion analysis system. Angular displacement of the upper vertebra (C-3) in relation to the lower vertebra (C-4) was calculated from marker position by using custom-made computer software. The experimental error associated with this method was ± 0.1°.[18]

The vertebrae were mounted in pots by using polymethylmethacrylate. The lower pot was rigidly attached to the base of the testing apparatus. This test setup resulted in a compressive preload of 25 N because of the weight of the upper fixation pot, which represents the mean weight of the head of the sheep. Moments were applied in a quasistatic manner in increments of 1 Nm to a maximum of 6 Nm. Specimens were preconditioned with three cycles of 6-Nm load with a velocity of 1.2 mm/second of the transverse bar. The fourth cycle was measured.

The mean apparent stiffness values in the EZ were calculated from the corresponding load-displacement curves. Range of motion, NZ, and EZ were determined.

Histomorphological, Histomorphometric, and Fluorochrome Analyses

All C3–4 motion segments were harvested at 12 weeks for histological examination of the bone. The motion segments had been fixed for 7 days in 10% normal buffered formaldehyde followed by dehydration in ascending concentrations of ethanol and embedded without being decalcified, in methylmethacrylate.

For histomorphological and histomorphometric analyses longitudinal sections in the sagittal plane were cut at 6 μm by using a microtome and a 40° stainless-steel knife. The residual parts of the cages were then removed, and the following stains were used: safranin O/lightgreen, Safranin O/van Kossa, astrablue, and Masson–Goldner. Masson–Goldner staining was used for histomorphological analysis.

Histomorphological analysis included evaluation of bone fusion according to the A through D parameters previously defined. The maximum intervertebral gap in craniocaudal direction was measured directly on midsagittal sections.

Histomorphometric parameters were measured on the residual stainings using a Leica microscope and the image analyzing system. Parameters were measured at a magnification of ×1.6. The sagittal diameter distance of C-3 and the mean preoperative DSH were determined to define the size of the ROI for histomorphometric evaluation (Fig. 3). The complete intervertebral fusion area was included in this ROI. The following structural indices were calculated in the ROI: bone volume/total volume, cartilage volume/total volume, and mineralized cartilage volume/cartilage volume.

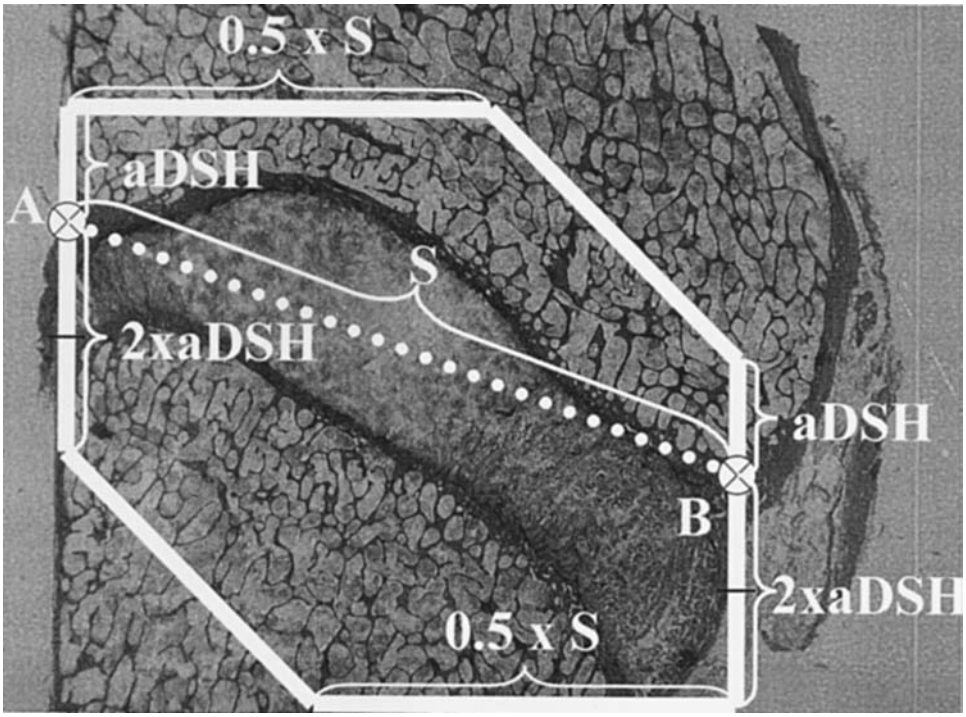


FIG. 3. Region of interest for histomorphometric evaluation. The ROI for histomorphometric evaluation was determined using the points A (posterior edge of the C-3 endplate) and B (anterior edge of the C-3 endplate) and the preoperative average DSH (aDSH) evaluated on lateral x-ray films of the C3–4 motion segment. The distance between A and B was defined as the sagittal diameter distance (S). From points A and B a line was drawn with a distance of one aDSH in cranial and two aDSH (2×aDSH) in caudal direction. From the resulting upper and lower endpoints, an additional horizontal line was drawn with a distance of half S (0.5×S). Interconnecting the resultant endpoints leads to a hexagonal structure representing the complete ROI for histomorphometic evaluation.

For fluorochrome analysis longitudinal sections in the parasagittal plane were cut at 400 μm with a precise macrogrinding machine. These slices were then ground to a thickness of 80 μm by using a precise microgrinding machine. Fluorochrome markers were analyzed under appropriate lighting conditions by using a Leica microscope and an image analyzing system. Parameters were measured at a magnification of ×1.6.

Fluorochrome analysis of intervertebral fusion areas has previously been described in detail.[38] The first appearance of the marker served to indicate formation of new bone matrix. The presence or absence of each marker around or within the cage or the bone graft, respectively, was used to determine the relative time frame of new bone formation.

Sources of Equipment

We obtained the Harms cages from Motech GmbH (Schwenningen, Germany) and the SynCage-3 implants from Synthes GmbH (Bochum, Germany). The radiography unit (Mobilett Plus), the quantitative CT scanner (Somatom plus 4), and scanner software (Sienet MagicView VA 30A) were purchased from Siemens AG (Erlangen, Germany). We acquired the x-ray film (CR 24X30) from Fuji (Kleve, Germany). The macro- and microgrinding machines were manufactured by Fa. Exact (Norderstedt, Germany). The device for measuring Newtons was obtained from Inha GmbH (Berlin, Germany). The imaging analysis system (model KS 400) used to measure BCV was produced by Zeiss GmbH (Oberkochen, Germany).

We acquired the uniaxial testing machine (model 1456) from Zwick GmbH (Ulm, Germany). The optical measurement system, the three-diode triangular markers, and the computerized motion analysis system (PC-Reflex) were purchased from Qualysis (Sävebalden, Sweden). We acquired the polymethylmethacrylate (Technovit 3040) and the methylmethacrylate (Technovit 9100) from Heraeus Kulzer GmbH (Wehrheim/Ts, Germany). The SPSS software was obtained from SPSS (Chicago, IL).

Statistical Analysis

Comparison of data was performed using one-way analysis of variance for independent samples followed by Tukey post-hoc analysis for multiple comparison procedures in which Bonferroni correction was used for multiple measurements. Intraobserver variability for radiographic, functional radiographic evaluation and CT measurements was determined using κ statistics. The A through D scores previously described (see *Radiographic Evaluation*) used to categorize semiquantitative bone fusion on plain radiographs, CT scans, and histological stainings; however, no statistical evaluation of this score was performed. Statistically significant differences were defined at a 95% confidence level. The values are given as mean ± standard deviation. The SPSS software supported statistical evaluation.

Results

Failure Parameters and Complications

One sheep died of an anesthesia-related complication on Day 0. This animal was excluded from the study and replaced by another animal.

In Group 1, one sheep developed a hematoma at the donor site of the iliac crest graft. In group 3, one sheep developed wound healing problems at the donor site. Both

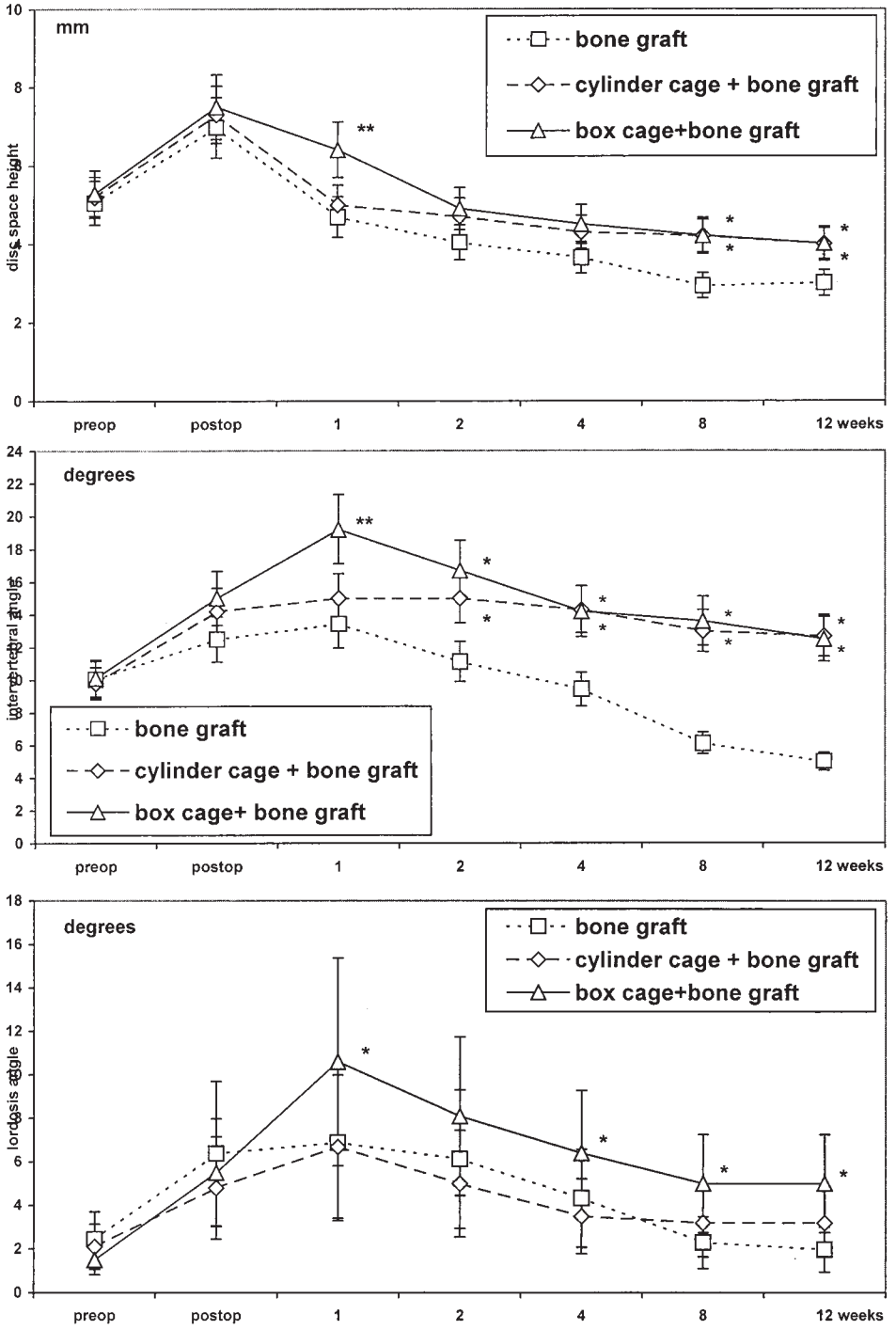


FIG. 4. Radiographic analyses. *Upper:* Mean DSH of the different groups throughout the observation period. *$p < 0.05$ compared with the bone graft. **$p < 0.05$ compared with the bone graft and the cylinder-design cage. *Center:* Mean IVA of the different groups throughout the observation period. *$p < 0.05$ compared with the bone graft. **$p < 0.05$ compared with the bone graft and the cylinder-design cage. *Lower:* Mean LA of the different groups throughout the observation period. *$p < 0.05$ compared with the bone graft.

complications resolved without further difficulty under provision of conservative treatment.

Volume of the Implants

The volume of the implants was evaluated prior to insertion by using water-displacement technique. The mean volume of the autologous tricortical iliac crest bone graft was 1.3 ± 0.1 cm^3. The mean volumes of the autologous iliac crest graft–filled cylinder- and box-design cages were 1.52 ± 0.1 and 1.5 ± 0.1 cm^3, respectively.

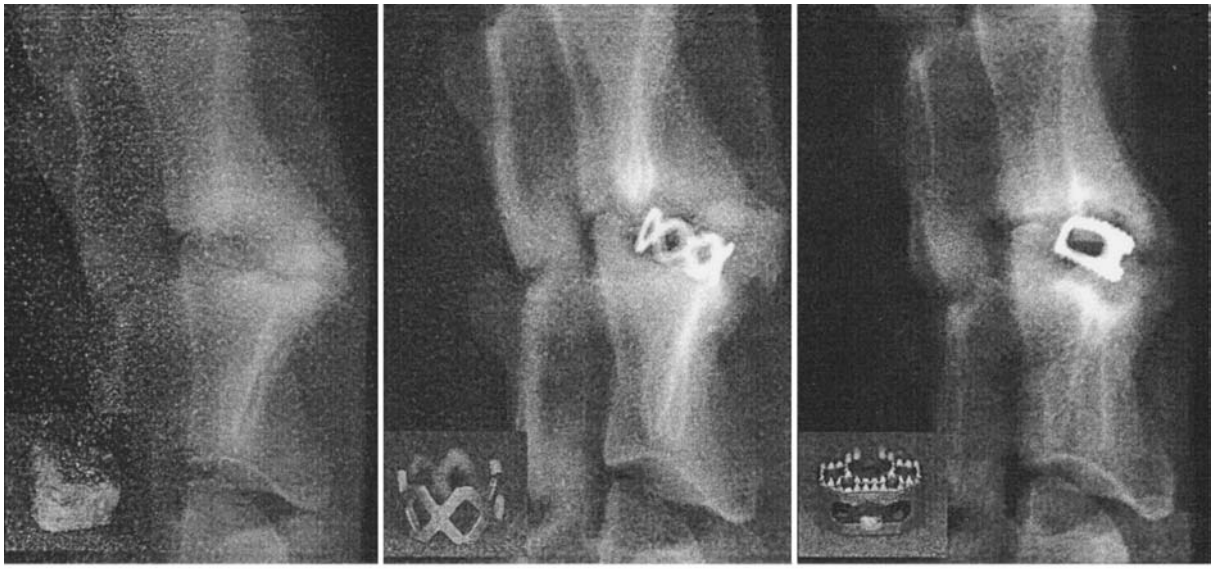


FIG. 5. Radiographic analysis. After 12 weeks, the extent of osseous fusion was evaluated on radiographs. The small pictures within the radiographs show the different implants. Notice the larger contiguous cranial pore in the cylinder-design cage. The results of the fusion score are depicted: A, no osseous fusion; B, maximum intervertebral gap of more than 5 mm; C, maximum intervertebral gap of less than 5 mm; and D, complete osseous fusion.

Radiographic Results

We conducted repeated measurements to determine the reproducibility of the positioning of the cervical spines in the fixation devices for radiographic evaluation. The reproducibility of the mean DSH and IVA was high, showing a maximum difference on 10 radiographs of 0.8 mm (approximately 10% of total value) and 1.5° (approximately 10% of total value), respectively. The reproducibility of the LA, however, was moderate showing a maximum difference on 10 radiographs of 4° (approximately 60% of total value).

Intraobserver agreement for radiographic measurements was good, showing κ values ranging from 0.76 to 0.92.

With regard to preoperative baseline values of all radiographic parameters, there were no intergroup differences. At 8 and 12 weeks both cage-fitted groups showed significantly higher values for mean DSH than Group 1 ($p < 0.05$; Fig. 4 *upper*). At 1 week mean DSH values for the box-design cage were significantly higher than those for the cylinder-design cage and the tricortical iliac crest bone graft ($p < 0.05$); however, no differences were found in DSH between the two cage types during the experimental period. At 2, 4, 8, and 12 weeks both cage-treated groups (Groups 2 and 3) showed significantly higher values for IVA ($p < 0.05$; Fig. 4 *center*) compared with the autologous tricortical iliac crest graft–treated group (Group 1). During the experimental period, no significant differences in IVA were found between the cage groups, except for at the 2-week time point, at which the box-design cage showed a significantly higher IVA than the cylinder-design cage ($p < 0.05$). At 1, 4, 8, and 12 weeks the box-design cage (Group 3) showed significantly higher values for LA ($p < 0.05$; Fig. 4 *lower*) compared with the autologous iliac crest graft group (Group 1) and the cylinder-design cage group (Group 2).

After 12 weeks, bone fusion was evaluated radiographically scans (Fig. 5). In the cage groups (Groups 2 and 3), a slightly more advanced interbody fusion was found compared with that in Group 1 (Table 1).

Functional Radiographic Results

Intraobserver agreement with regard to functional radiographic measurements was excellent, showing κ values ranging from 0.84 to 0.96.

Flexion–extension differences in LA were not significantly different between Groups 2 and 3 (Table 2); however, functional radiographic assessment revealed significantly lower residual flexion–extension movement in the cylinder-design cage group (Group 2) than in the tricortical iliac crest graft group (Group 1) ($p < 0.05$).

Quantitative CT Results

Intraobserver agreement for CT measurements was excellent, showing κ values ranging from 0.82 to 0.92.

After 12 weeks, there were no significant differences for BMD between the tricortical iliac crest graft group (Group 1) and both cage groups. In the cylinder-design

TABLE 1

*Radiographic fusion results after 12 weeks**

Fusion Parameter (score)	No. of Specimens		
	Group 1 (bone graft)	Group 2 (cylinder cage)	Group 3 (box cage)
A	0	0	0
B	5	4	5
C	3	3	3
D	0	1	0

* See *Radiographic Evaluation* for definition of scores.

TABLE 2

Summary of functional radiographic flexion–extension data after 3 weeks

Flexion/ Extension	Difference in Degrees (range)		
	Group 1	Group 2	Group 3
IVA	13.6 ± 3.7 (6.0–13.5)	7.7 ± 3.0* (4.5–11.0)	9.6 ± 2.8 (7.0–12.5)
LA	14.1 ± 5.0 (6.5–17.0)	7.9 ± 3.4* (4.5–11.5)	10.1 ± 3.2 (6.0–13.5)

* $p < 0.05$ compared with Group 1.

cage group (Group 2) BMC of the callus was significantly higher than that in Groups 1 and 3 ($p < 0.05$). The cylinder-design cages (Group 2) showed significantly higher values for BCV ($p < 0.05$) than the other groups (Fig. 6, Table 3).

Bone fusion was evaluated on 2D CT reconstructions (Table 4). In the two cage groups a slightly more advanced interbody fusion was found compared with that in Group 1 (Table 1).

Biomechanical Results

Biomechanical results for stiffness, ROM, NZ, and EZ are depicted in Fig. 7 and Table 5.

The highest stiffness values and the lowest ROM, NZ, and EZ values in rotation and bending were consistently found in Group 2. The mean stiffness value in axial rotation and lateral bending was significantly higher ($p < 0.05$) in the cylinder-design cages than in the other groups. The ROM in rotation was significantly lower ($p < 0.05$) in Group 2 than in the other groups.

Histomorphological Results

Histomorphological analysis supported the findings of radiographic and biomechanical examinations (Fig. 8).

The spines fixed with autologous iliac crest graft (Group 1) showed extensive callus formation with cartilage and connective tissue cells. The tricortical bone graft, however, collapsed and was mainly resorbed. Osteoclastic activity was noted as an indicator of graft resorption. Most of the callus was seen ventrally. Those spines fixed with the two types of cage (Groups 2 and 3), showed islands of bone between the endplates with cartilage and fibrous tissue components. These findings were accompanied by capillary ingrowth and resorptive lacunae. The tissue surrounding the cages appeared similar in both groups. Within the Group 3 cages, extensive osteoclastic activity was noted as an indication of graft resorption.

Bone fusion was evaluated histomorphologically (Table 6). In the two cage groups a more advanced interbody fusion mass was found compared with that in the tricortical iliac crest graft group. In the cylinder-design cages, however, a slightly more advanced bone matrix formation was shown than in the box-design cage.

Histomorphometric Results

The results of histomorphometric analysis are summarized in Table 7. No significant differences in sagittal diameter distance (baseline) were determined among all groups. Compared with the bone graft group and the box-design cage group histomorphometric parameters showed significantly more advanced bone formation (bone volume/total volume) in the cylinder-design cage group ($p < 0.05$). The bone graft–stabilized group showed significantly higher histomorphometric values in cartilage volume/total volume and mineralized cartilage volume/total volume than both cage groups ($p < 0.05$).

Fluorochrome Analysis Results

The results of fluorochrome analysis are summarized in Table 8. In spines stabilized with both cage types earlier new bone formation was exhibited both within and around the cages compared with those stabilized with the bone graft group at all time points. Compared with the box-design cage, the cylinder-design cage showed a greater amount of new bone formation both within and around the cages at 6 and 9 weeks.

Discussion

The objective of this study was to compare characteristics of interbody fusion among an autologous tricortical iliac crest bone graft, a cylinder-design cage, and a box-design cage in a sheep cervical spine interbody fusion model. This study was designed to determine whether there were differences among the three interbody fusion materials with regard to: 1) the ability to preserve postoperative distraction; 2) biomechanical stability; and 3) histological characteristics of intervertebral bone matrix formation.

Distractive Properties

The authors of several clinical studies have demonstrated that tricortical iliac crest graft–assisted interbody fusion induced decreases in DSH during the postoperative period.[8,10,20] The findings in these clinical investigations are in agreement with the experimental results in our study, with regard to a significant decrease in DSH in the bone graft group. Interbody cages have been specially developed in the quest to provide interspace structural stability during bone fusion. Cages should retain interbody distraction and should be resistant to subsidence into the adjacent vertebrae to guarantee the desired quality of fu-

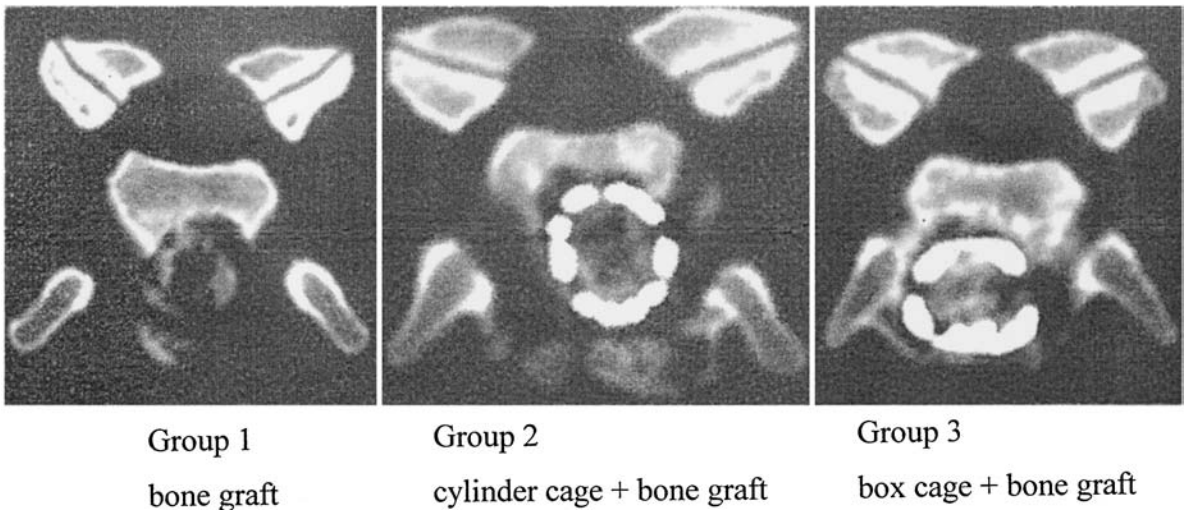


FIG. 6. Computerized tomography analysis. After 12 weeks, interbody fusion was evaluated on axial CT scans obtained parallel to the intervertebral space.

sion.[37] Currently, however, subsidence of cages has only been investigated in one animal experimental in vivo study. Sandhu, et al.,[34] demonstrated in a sheep model that screw-design cages preserved interbody distraction more effectively than an autologous iliac crest bone graft. Experimental in vivo data for cylinder- or box-design cages are not currently available. Analysis of the data in the present study demonstrated that, at the time of surgery, both cages and the tricortical iliac crest graft were able to distract intervertebral spaces beyond their baseline measurements to nearly the same values. In all conditions, however, significant subsidence developed beyond normal DSH values during the 12-week observation period. Whereas the loss of DSH of the cages resulted from subsidence into the subchondral bone of the adjacent vertebral bodies, the loss of the intervertebral space in the tricortical iliac crest graft group resulted mainly from gradual graft collapse. Both cages were able to decrease the loss of DSH significantly compared with the tricortical iliac crest graft. Although the box-design cage showed significantly less subsidence after 2 weeks, both cages demonstrated similar DSH values at final measurements.

TABLE 3

Summary of quantitative CT data for BMD, BMC, and BCV after 12 weeks

Quantitative Scanning	Group 1	Group 2	Group 3
BMD (g/cm^3)	0.58 ± 0.04 (0.56–0.64)	0.55 ± 0.05 (0.52–0.59)	0.58 ± 0.07 (0.52–0.59)
BMC (g)	2.3 ± 1.0 (1.7–3.1)	3.2 ± 0.3†‡ (2.8–3.7)	2.2 ± 0.3 (1.8–3.6)
BCV (cm^3)	4.0 ± 1.0* (3.8–5.1)	5.4 ± 1.4*†‡ (3.8–6.3)	3.8 ± 0.3* (3.0–4.4)

* Initial bone graft volumes of the iliac crest graft, the cylinder cage, and box cage were 1.3, 1.5, and 1.52 cm^3, respectively.
† $p < 0.05$ compared with Group 1.
‡ $p < 0.05$ compared with Group 3.

Biomechanical Properties

In this in vivo experiment the tricortical iliac crest bone graft has shown significantly less biomechanical stiffness in bending and rotation and a higher ROM in rotation than the cylinder-design cage. These in vivo results are in accordance with those reported in several in vitro studies; a higher biomechanical stiffness was demonstrated for the cylinder-design cage used in this study than for a bone graft.[22,26] In a previous biomechanical in vitro study reported by Mittlmeier, et al.,[26] the authors found that a significantly higher biomechanical stiffness was associated with the box-design cage than with the tricortical bone graft. In contrast to the findings of Mittlmeier, et al.,[26] we found no significant biomechanical difference between the box-design cage and the bone graft after 12 weeks in vivo. Additionally, we found a higher biomechanical stiffness in rotation and bending and a lower ROM in rotation for the cylinder-design cage than for the box-design cage. These in vivo results are also in crucial contrast to in vitro results obtained by our own working group.[26] In our previous in vitro studies,[26] we demonstrated significantly higher stiffness for the box-design cage than for the cylinder-design cage. The differences between biomechanical in vitro and in vivo results may be a result of the "biological qualities"

TABLE 4

Summary of fusion results observed on 2D CT reconstructions after 12 weeks

Fusion Parameter (score)	No. of Specimens		
	Group 1	Group 2	Group 3
A	0	0	0
B	5	4	6
C	3	3	2
D	0	1	0

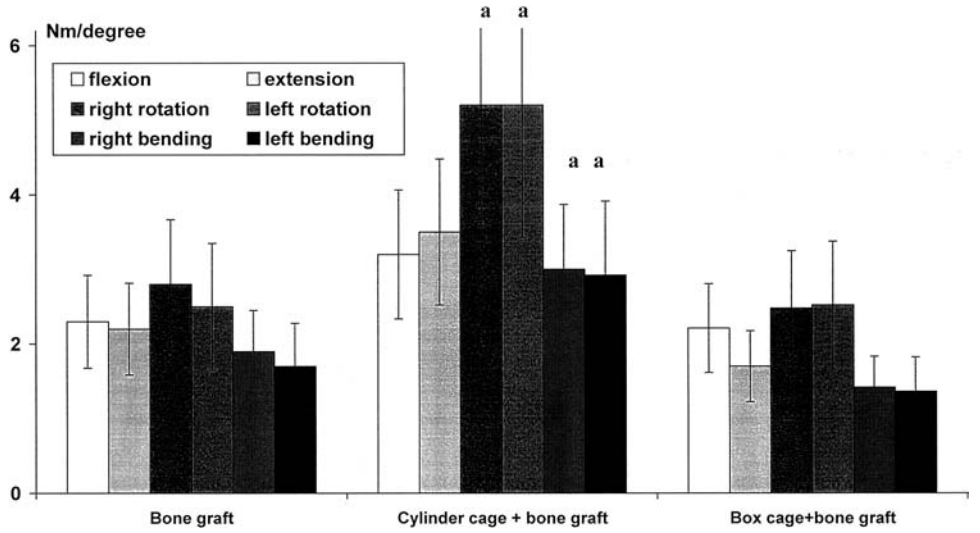


FIG. 7. Bar graph demonstrating results of biomechanical stiffness of the different groups for the different test modes. a = $p < 0.05$ compared with the bone graft (Group 1) and the box-design cage (Group 3).

of different cage designs in vivo, suggesting that currently available biomechanical in vitro tests are not highly predictive of the in vivo performance of any interbody fusion cage. The predictive value of biomechanical in vitro tests for the in vivo performance of spinal implants has also been questioned by other authors.[6,22,26,32,36]

Histological Characteristics

Histomorphometric analysis demonstrated the presence of significantly higher intervertebral bone volume in the cylinder-design cage group than in the bone graft group. Additionally, significantly higher values for bone matrix formation were found in the cylinder design-group than in the box-design group. There are some possible explanations for these findings.

The limited biomechanical properties of the tricortical iliac crest bone graft resulted in a compression and sometimes fragmentation of the graft, which was followed by extensive osteoclastic activity and finally graft resorption. In contrast, because the bone grafts packed inside the cages have a biomechanically protected and, consequently, biologically improved environment for fusion,[37] a more stable interbody fusion mass develops, especially in the cylinder-design cage group.

The initial biomechanical in vivo stability of the cage combined with the graft composite is nearly exclusively a result of the biomechanical properties of the cage.[15,26] Only secondarily, the interbody fusion mass arising from the incorporated bone graft contributes to biomechanical stability.[15] With the biomechanical in vitro results of both cages in mind,[26] the significantly greater biomechanical stability in the cylinder cage–fixed group compared with the box cage–fixed group in vivo was an effect of an accelerated interbody fusion in the former. Biomechanical loads in vivo consist of shear and compression. Whereas shear loads promote bone matrix formation, compressive loads do not.[5,7,30] In contrast to the bone graft, the cage mainly functions as a protector against compressive loads. Therefore, this cage-related factor contributed to the more stable interbody fusion mass in the cylinder-design cage group compared with the bone graft group.

The ideal biological environment for bone fusion is achieved by optimum grafting techniques and the maximum filling of the intervertebral space with graft material.[37] As cage volume increases, however, graft volume

TABLE 5

*Summary of biomechanical results after 12 weeks**

Test mode (degrees)	Group 1	Group 2	Group 3
flexion			
ROM	2.9 ± 1.0	3.9 ± 1.3	3.9 ± 1.4
NZ	0.7 ± 0.5	1.3 ± 0.9	1.6 ± 1.0
EZ	1.9 ± 0.7	2.6 ± 0.8	2.3 ± 0.9
extension			
ROM	3.3 ± 1.5	3.8 ± 1.2	3.9 ± 1.4
NZ	0.9 ± 0.7	1.4 ± 0.9	1.7 ± 1.0
EZ	2.4 ± 0.8	2.4 ± 1.0	2.3 ± 1.0
right rotation			
ROM	3.3 ± 1.4	2.0 ± 1.0†‡	2.9 ± 0.8
NZ	0.7 ± 0.3	0.4 ± 0.2	0.6 ± 0.3
EZ	2.7 ± 1.1	1.6 ± 0.9†	2.3 ± 0.9
left rotation			
ROM	3.3 ± 1.4	2.1 ± 1.0†‡	2.9 ± 0.9
NZ	0.7 ± 0.5	0.5 ± 0.3	0.6 ± 0.3
EZ	2.7 ± 1.8	1.6 ± 0.9	2.3 ± 0.7
right bending			
ROM	4.5 ± 2.4	4.2 ± 2.2	5.6 ± 2.2
NZ	1.5 ± 1.5	1.2 ± 1.2	1.9 ± 1.0
EZ	3.0 ±1.3	3.0 ± 1.9	3.7 ± 1.8
left bending			
ROM	4.6 ± 2.2	4.1 ± 2.1	5.6 ± 2.2
NZ	1.7 ± 1.6	1.3 ± 1.2	2.0 ± 1.3
EZ	2.9 ± 0.7	2.8 ± 1.9	3.6 ± 1.8

* All values are presented in degrees.
† $p < 0.05$ compared with Group 1.
‡ $p < 0.05$ compared with Group 3.

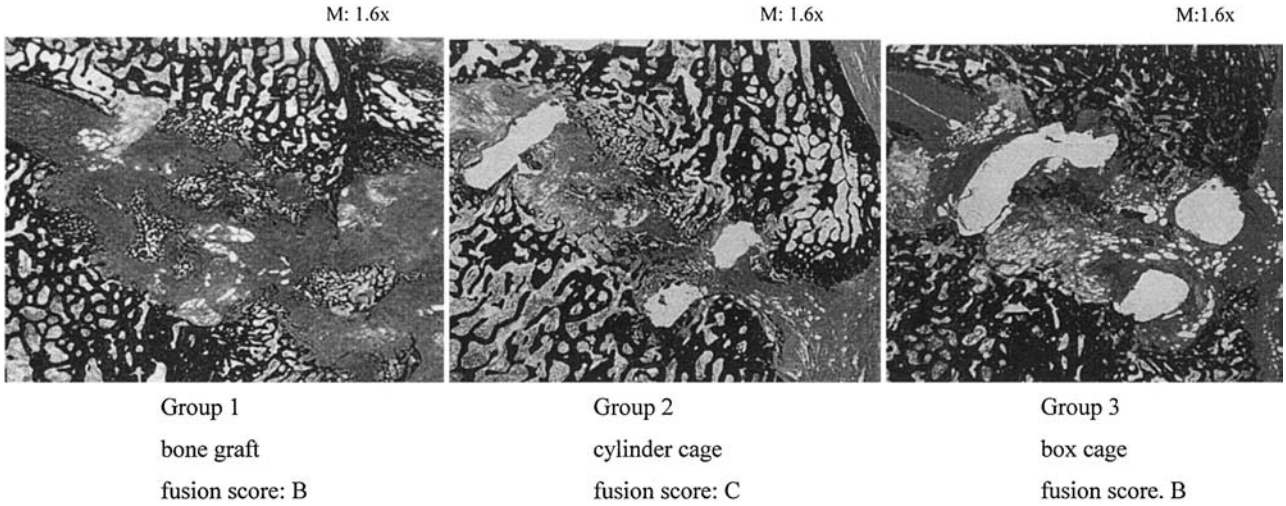


FIG. 8. Histomorphological analysis. After 12 weeks, interbody fusion was evaluated histomorphologically and histomorphometrically. Safranin-O/v. Kossa staining, original magnification (M) × 1.6.

decreases. Therefore, biologically, the ideal cage would be the one with the smallest cage volume that will provide adequate mechanical stability, because this cage will allow for the maximum filling of the intervertebral space with graft material.[37] Although both cages in this study were of similar volumes when filled with bone grafts (cylinder-design cage: 1.52 cm^3; box-design cage: 1.50 cm^3), the volume of the isolated cages was substantially different (cylinder-design cage: 0.1 cm^3; box-design cage: 0.26 cm^3). Therefore, a higher bone graft volume was incorporated in the cylinder-design cage, which potentially contributed to the more stable intervertebral fusion mass in this group.

Kanayama, et al.,[15] in an in vitro study, showed that the cage design has a significant influence on the loads of a graft within a cage. They demonstrated that a larger contiguous pore is important to decrease the stress-shielding effect on a graft within an interbody fusion device. The authors assumed that the lower the stress-shielding effect on a graft, the greater the possibility for interbody fusion. Finally, they wrote that "it remains unclear whether the stress-shielding environment influences the bone quality of the developing interbody fusion mass" in vivo.[15] The incorporation and remodeling of a bone graft within an interbody cage has been investigated in several animal experiments. Brantigan, et al.,[1] placed carbon cage implants in a goat model and found a complete incorporation of the autograft and continuous trabecular bridging within the cage. Cunningham, et al.,[9] and Zdeblick, et al.,[38] investigated the efficacy of a screw-design cage in a sheep model. They found no significant difference in bone quality of the interbody fusion mass between the autologous bone–packed screw-design cage and the tricortical iliac crest graft during a short-term postoperative observation period. In the present study we evaluated bone matrix formation within both cages. A significantly higher interbody bone volume and an accelerated interbody fusion on polychrome sequential labeling was shown in the cylinder-design cages than in the box-design cage. Therefore, it can be assumed that whereas both cage designs were able to provide an adequate biological environment for interbody fusion, the cylinder-design cage was more effective than the box-design cage. Comparing both cages, a significantly larger contiguous pore was obvious in the cylinder-design cage (Fig. 5). Therefore, based on the results reported by Kanayama, et al.,[15] the cylinder-design cage apparently has a significant lower stress-shielding effect on the incorporated bone graft than the box-design cage.

TABLE 6

Summary of fusion results determined by histomorphological analysis after 12 weeks

Fusion Parameter (score)	Group 1	Group 2	Group 3
A	0	0	0
B	5	4	5
C	3	3	3
D	0	1	0

TABLE 7

*Summary of histomorphometric results obtained after 12 weeks**

Index	Group 1	Group 2	Group 3
baseline SDD (mm)	26.2 ± 1.0 (25.0–28.3)	25.5 ± 1.1 (24.6–27.8)	26.1 ± 0.8 (25.2–28.0)
BV/TV (%)	31.4 ± 3.9 (24.8–39.0)	45.5 ± 6.7†‡ (38.5–61.3)	31.6 ± 3.8 (20.5–42.3)
CV/TV (%)	10.1± 2.8 (1.0–22.1)	4.6 ± 2.7† (0.8–9.4)	4.2 ± 1.1† (2.8–6.4)
mCV/CV (%)	5.5 ± 2.0 (0.6–9.4)	2.8 ± 1.3† (0.2–7.6)	3.2 ± 1.4† (1.0–5.1)

* Initial graft volumes of the iliac crest graft, the cylinder cage, and box cage were filled 1.3, 1.5, and 1.52 cm^3, respectively. Abbreviations: BV/TV = bone volume/total volume; CV/TV = cartilage volume/total volume; mCV/TV = mineralized CV/TV; SDD = sagittal diameter distance.
† $p < 0.05$ compared with Group 1.
‡ $p < 0.05$ compared with Group 3.

TABLE 8

*Summary of results of fluorochrome analysis after 12 weeks**

	Group 1		Group 2		Group 3	
Index	Adjacent	W/in	Adjacent	W/in	Adjacent	W/in
3 wks	0	0	0	1	1	0
6 wks	1	0	4	6	2	4
9 wks	1	0	4	6	4	4

* Depicted are the number of fusion sites (of the different groups at different time points) in which the fluorochrome marker was present adjacent to or within the cage or bone graft, respectively.

In conclusion, the significantly lower stress-shielding effect of the bone graft within the cylinder-design cage might be the most important cause of the significantly higher interbody fusion mass found in this cage design.

Conclusions

Compared with the tricortical bone graft, both cage designs were associated with significantly better distractive properties. A significantly greater biomechanical stiffness in rotation and bending and an accelerated interbody fusion was associated with cylinder-design cages than the box-design cage and the tricortical bone graft. The differences in bone matrix formation within both cages were mainly a result of the significantly lower stress-shielding effect of the bone graft within the cylinder-design cage. Further investigations are necessary to determine quantitatively the small borderline between biomechanically protected environment of the graft within a cage that accelerates interbody fusion and the stress shielding of a graft within the cage that inhibits interbody fusion.

References

1. Brantigan JW, McAfee PC, Cunningham BW: Interbody lumbar fusion using a carbon fiber implant versus allograft bone. An investigational study in the Spanish goat. **Spine 19:** 1436–1444, 1994
2. Brantigan JW, Steffee AD, Geiger JM: A carbon fiber implant to aid interbody lumbar fusion. Mechanical testing. **Spine 16:** S277–S282, 1991
3. Brodke DS, Dick JC, Kunz DN, et al: Posterior lumbar interbody fusion. A biomechanical comparison, including a new threaded cage. **Spine 22:**26–31, 1997
4. Brooke NS, Rorke AW, King AT, et al: Preliminary experience of carbon fibre cage prostheses for treatment of cervical spine disorders. **Br J Neurosurg 11:**221–227, 1997
5. Burger EH, Klein-Nulend J, Veldhuijzen JP: Mechanical stress and osteogenesis in vitro. **J Bone Miner Res 7:**327–401, 1992
6. Cain CC, Fraser RD: Bony and vascular anatomy of the normal cervical spine in the sheep. **Spine 20:**759–765, 1995
7. Carter DR, Wong M: Mechanical stresses and endochondral ossification in the chondroepiphysis. **J Orthop Res 6:**148–154, 1988
8. Cloward RB: The anterior surgical approach to the cervical spine: the Cloward Procedure: past, present, and future. The presidential guest lecture, Cervical Spine Research Society. **Spine 13:**823–827, 1988
9. Cunningham BW, Kanayama M, Parker LM, et al: Osteogenic protein versus autologous interbody arthrodesis in the sheep thoracic spine. A comparative endoscopic study using the Bagby and Kuslich interbody fusion device. **Spine 24:**509–518, 1999
10. Dennis S, Watkins R, Landaker S, et al: Comparison of disc space heights after anterior lumbar interbody fusion. **Spine 14:**876–878, 1989
11. Goh JC, Wong HK, Thambyah A, et al: Influence of PLIF cage size on lumbar spine stability. **Spine 25:**35–40, 2000
12. Heller JG, Zdeblick TA, Kunz DA, et al: Spinal instrumentation for metastatic disease: in vitro biomechanical analysis. **J Spinal Disord 6:**17–22, 1993
13. Hollowell JP, Vollmer DG, Wilson CR, et al: Biomechanical analysis of thoracolumbar interbody constructs. How important is the endplate? **Spine 21:**1032–1036, 1996
14. Jost B, Cripton PA, Lund T, et al: Compressive strength of interbody cages in the lumbar spine: the effect of cage shape, posterior instrumentation and bone density. **Eur Spine J 7:** 132–141, 1998
15. Kanayama M, Cunningham BW, Haggerty CJ, et al: In vitro biomechanical investigation of the stability and stress-shielding effect of lumbar interbody fusion devices. **J Neurosurg (Spine 2) 93:**259–265, 2000
16. Kandziora F, Kerschbaumer F, Starker M, et al: Biomechanical assessment of transoral plate fixation for atlantoaxial instability. **Spine 25:**1555–1561, 2000
17. Kandziora F, Mittlmeier T, Kerschbaumer F: Stage-related surgery for cervical spine instability in rheumatoid arthritis. **Eur Spine J 8:**371–381, 1999
18. Kandziora F, Pflugmacher R, Scholz M, et al: Comparison between sheep and human cervical spines. **Spine 26:**1028–1037, 2001
19. Kettler A, Wilke HJ, Dietl R, et al: Stabilizing effect of posterior lumbar interbody fusion cages before and after cyclic loading. **J Neurosurg (Spine 1) 92:**87–92, 2000
20. Kumar A, Kozak JA, Doherty BJ, et al: Interspace distraction and graft subsidence after anterior lumbar fusion with femoral strut allograft. **Spine 18:**2393–2400, 1993
21. Kumaresan S, Yoganandan N, Pintar FA: Finite element analysis of anterior cervical spine interbody fusion. **Biomed Mater Eng 7:**221–230, 1997
22. Lee SW, Lim TH, You JW, et al: Biomechanical effect of anterior grafting devices on the rotational stability of spinal constructs. **J Spinal Disord 13:**150–155, 2000
23. Lund T, Oxland TR, Jost B, et al: Interbody cage stabilisation in the lumbar spine: biomechanical evaluation of cage design, posterior instrumentation and bone density. **J Bone Joint Surg (Br) 80:**351–359, 1998
24. Majd ME, Vadhva M, Holt RT. Anterior cervical reconstruction using titanium cages with anterior plating. **Spine 24:**1604–1610, 1999
25. Matge G: Anterior interbody fusion with the BAK-cage in cervical spondylosis. **Acta Neurochir (Wien) 140:**1–8, 1998
26. Mittlmeier T, Pflugmacher R, Schäfer J, et al: Cervical interbody fusion cages. Biomechanical comparison of screw, box and cylinder designs. **Europ Spine J 9:**297–298, 2000
27. Munoz FLO, de las Heras BG, Lopez VC, et al: Comparison of three techniques of anterior fusion in single-level cervical disc herniation. **Eur Spine J 7:**512–516, 1998
28. Nibu K, Panjabi MM, Oxland T, et al: Multidirectional stabilizing potential of BAK interbody spinal fusion system for anterior surgery. **J Spinal Disord 10:**357–362, 1997
29. Oxland TR, Hoffer Z, Nydegger T, et al: A comparative biomechanical investigation of anterior lumbar interbody cages: central and bilateral approches. **J Bone Joint Surg (Am) 82:** 383–393, 2000
30. Patwardhan AG, Havey RM, Ghanayem AJ, et al: Load-carrying capacity of the human cervical spine in compression is increased under a follower load. **Spine 25:**1548–1554, 2000
31. Pitzen T, Caspar W, Matthis D, et al: [Primary stability of 2 PLIF (posterior lumbar interbody fusion) -a biomechanical in

vitro comparison.] **Z Orthop Ihre Grenzgeb 137:**214-218, 1999 (Ger)
32. Rapoff AJ, Ghanayem AJ, Zdeblick TA: Biomechanical comparison of posterior lumbar interbody fusion cages. **Spine 22:** 2375–2379, 1997
33. Rathonyi GC, Oxland TR, Gerich U, et al: The role of supplemental translaminar screws in anterior interbody fixation: a biomechanical study. **Eur Spine J 7:**400–407, 1998
34. Sandhu HS, Turner S, Kabo M, et al: Distractive properties of threaded interbody fusion device. An in vivo model. **Spine 21:** 1201–1210, 1996
35. Tencer AF, Hampton D, Eddy S: Biomechanical properties of threaded inserts for lumbar interbody spinal fusion. **Spine 20:** 2408–2414, 1995
36. Volkman T, Horton WC, Hutton WC: Transfacet screws with lumbar interbody reconstruction: biomechanical study of motion segment stiffness. **J Spinal Disord 9:**425–432, 1996
37. Weiner BK, Fraser RD: Spine update lumbar interbody fusion cages. **Spine 23:**634–640, 1998
38. Zdeblick TS, Ghanayem AJ, Rapoff AJ, et al: Cervical interbody fusion cages. An animal model with and without bone morphogenetic protein. **Spine 23:**758–766, 1998

Manuscript received April 23, 2001.
Accepted in final form November 15, 2001.
Address reprint requests to: Frank Kandziora, M.D., Unfall- und Wiederherstellungschirurgie, Universitätsklinikum Charité der Humboldt Universität Berlin, Campus Virchow-Klinikum, Augustenburgerplatz 1, 13353 Berlin, Germany. email: frank.kandziora@charite.de.

Diese Untersuchung konnte nachweisen, dass mit Spongiosa gefüllte Cages im Vergleich zum trikortikalen Beckenkammspanimplantat in der Lage sind, die Ergebnisse der zervikalen Spondylodese zu verbessern. Die vorliegende Untersuchung konnte des weiteren zeigen, dass trotz standardisierter Operationsbedingungen Unterschiede im Einheilungsverhalten verschiedener Cagedesigns existieren.

2.2.3 Einfluss von Designparametern auf das Cage-Einheilungsverhalten

Da das Design von Cages offensichtlich eine wesentliche Bedeutung für die Einheilung der Implantate hat, sollte in dieser Untersuchung geklärt werden, welche Designparameter im Einzelnen Einfluss auf das Einheilungsverhalten ausüben. Als entscheidende Designparameter wurden bisher in in vitro Untersuchungen die Endplattenkonfiguration (Auflagefläche), die maximale Pore in der Auflagefläche und die in vitro Primärstabilität des Cages definiert [25,29,51,79,87,165]. Ziel dieser Untersuchung war es daher, folgende aus vorausgegangenen Untersuchungen hervorgegangene Hypothesen auf ihre Bedeutung zu prüfen:

- Je größer die Auflagefläche des Cages, desto geringer die Sinterung des Cages.
- Je größer die maximale Pore in der Auflagefläche eines Cages, desto geringer das „stress shielding“ auf die inkorporierte Spongiosa und desto besser das Einheilungsverhalten des Cages.
- Je höher die biomechanische Primärstabilität eines Cages in vitro, desto höher die biomechanische Sekundärstabilität in vivo.

Chirurg 2002 · 73:909–917
DOI 10.1007/s00104-002-0489-2

Originalia

F. Kandziora · R. Pflugmacher · M. Scholz · J. Schäfer · G. Schollmeier · K. J. Schnake
H. Bail · G. Duda · N. P. Haas
Unfall- und Wiederherstellungschirurgie, Campus Virchow-Klinikum, Universitätsklinikum Charité der Humboldt-Universität Berlin

Experimentelle Spondylodese der Schafshalswirbelsäule

Teil 1: Der Effekt des Cage-Designs auf die intervertebrale Fusion

Experimental fusion of the sheep cervical spine. Part I: Effect of cage design on interbody fusion

Abstract

Introduction. There has been a rapid increase in the use of interbody fusion cages as an adjunct to spondylodesis, although experimental data are lacking. A sheep cervical spine interbody fusion model was used to determine the effect of different cage design parameters (endplate-implant contact area, maximum contiguous pore) on interbody fusion.
Material and Method. *In vitro evaluation:* 24 sheep cadaver specimens (C2–C5) were tested in flexion, extension, axial rotation, and lateral bending with a nondestructive flexibility method using a nonconstrained testing apparatus. Four different groups were examined: (1) control group (intact) (n=24), (2) autologous tricortical iliac crest bone graft (n=8), (3) Harms cage (n=8), and (4) SynCage-C (n=8). *In vivo evaluation:* 24 sheep underwent C3/4 discectomy and fusion: group 1: autologous tricortical iliac crest bone graft (n=8), group 2: Harms cage filled with autologous cancellous iliac crest bone grafts (n=8), and group 3: SynCage-C filled with autologous cancellous iliac crest bone grafts (n=8). Radiographic scans were performed pre- and postoperatively and after 1, 2, 4, 8, and 12 weeks, respectively. At the same time points, disc space height (DSH), height index (HI), intervertebral angle (IVA), and endplate angle (EA) were measured. After 12 weeks the animals were killed and fusion sites were evaluated using biomechanical testing in flexion, extension, axial rotation, and lateral bending. Additionally, histomorphological and histomorphometrical analyses were performed.
Results. Over a 12-week period the cage groups showed significantly higher values for DSH, HI, IVA, and EA compared to the bone graft. In vivo stiffness was significantly higher for the tricortical iliac crest bone graft and Harms cage than in vitro stiffness. However, there was no difference between in vitro and in vivo stiffness of the SynCage-C. Histomorphometrical evaluation showed a more progressed bone matrix formation in the Harms cage group than in both other groups.
Conclusion. The parameter endplate-implant contact area was not able to determine subsidence of cages. In contrast, the maximum contiguous pore of a cage significantly correlates with interbody bone matrix formation inside the cage. Additionally, there was no correlation between in vitro and in vivo stiffness of interbody fusion cages. Therefore, biomechanical in vitro studies are not able to determine in vivo outcome of fusion cages. Animal experimental evaluations of interbody fusion cages are essential prior to clinical use.

Keywords

Cervical spine · Sheep · Animal model · Interbody fusion · Cages · Design · Biomechanics

Zusammenfassung

Einleitung. Die intervertebrale Spondylodese mit Spongiosa-augmentierten intervertebralen Cages findet zunehmende klinische Verbreitung, obwohl experimentelle Daten weitgehend fehlen. Ziel dieser Studie war es daher, in einem zervikalen Schafsmodell den Effekt von Designparametern (Auflagefläche, maximale Pore) auf das Einheilungsverhalten von Cages zu untersuchen.
Material und Methode. *In-vitro-Untersuchungen:* 24 Wirbelsäulenexplantate (C2–C5) von Merinoschafen wurden in Flexion, Extension, Rotation und Seitneigung mittels nichtdestruktiver Steifigkeitsmessung biomechanisch getestet. Die Präparate wurden (1) intakt (Kontrollgruppe; n=24) sowie nach Diskektomie C3/C4 und Stabilisierung mit (2) autologem trikortikalem Beckenkammspan (n=8), (3) Harmscage (n=8) und (4) Syncage-C (n=8) evaluiert. *In-vivo-Untersuchungen:* Bei 24 Merinoschafen wurde eine intervertebrale zervikale Fusion C3/C4 mit 3 verschiedenen Stabilisierungsverfahren (n=8) durchgeführt. Gruppe 1: autologer trikortikaler Beckenkammspan; Gruppe 2: Harmscage, gefüllt mit autologer Spongiosa; Gruppe 3: Syncage-C, gefüllt mit autologer Spongiosa. Prä- und postoperativ

Gefördert durch: MBI – Max Biedermann Institut der Steinbeis Stiftung (RS/KF-82042)

Dr. F. Kandziora
Unfall- und Wiederherstellungschirurgie, Universitätsklinikum Charité der Humboldt Universität Berlin, Campus Virchow Klinikum, Augustenburgerplatz 1, 13353 Berlin, E-Mail: frank.kandziora@charite.de

sowie nach 1, 2, 4, 8 und 12 Wochen wurden Röntgenbilder angefertigt anhand derer Intervertebralwinkel, Grund-Deckplattenwinkel sowie Bandscheibenraumhöhen und intervertebraler Höhenindex vermessen wurden. Nach 12 Wochen wurden die Tiere getötet und biomechanische Testungen in Flexion/Extension, Rotation und Neigung durchgeführt, um die Steifigkeit des Bewegungssegmentes zu ermitteln. Histomorphologische und histomorphometrische Untersuchungen wurden vorgenommen.

Ergebnisse. Im Vergleich zum trikortikalen Beckenkammspan waren beide Cages in der Lage, die Höhe des Bandscheibenraums und die Lordosierung des Bewegungssegmentes signifikant besser zu erhalten. In vitro zeigte der Syncage-C, in vivo der Harmscage die signifikant höchste biomechanische Steifigkeit. Die Unterschiede zwischen In-vitro- und In-vivo-Steifigkeit waren für den Beckenkammspan und den Harmscage signifikant, jedoch nicht für den Syncage-C. Der Harmscage zeigte histomorphometrisch ein signifikant beschleunigtes Einheilungsverhalten im Vergleich zum Syncage-C.

Schlussfolgerung. Die Auflagefläche eines Cages hat für das Sinterungsverhalten des Implantats in vivo nur eine untergeordnete Bedeutung. Hingegen weist die Größe der maximalen Pore eines spongiosagefüllten Cages eine positive Korrelation zum Einheilungsverhalten des Implantates auf. Da kein Zusammenhang zwischen der In-vitro-Primärstabilität und der In-vivo-Sekundärstabilität von intervertebralen Implantaten in dieser Studie bestand, sind biomechanische In-vitro-Untersuchungen nicht dazu geeignet, das Einheilungsverhalten eines Cages zu prognostizieren. Daher ist eine tierexperimentelle In-vivo-Evaluation neuer Cagedesigns vor deren Markteinführung zu fordern.

Schlüsselworte

HWS · Schaf · Tiermodel · Cage · Intervertebrale Fusion · Designparameter · Biomechanik

Der trikortikale Beckenkammspann war jahrzehntelang das Implantat der Wahl zur ventralen interkorporellen Spondylodese der Halswirbelsäule [4, 16]. Die Entnahme des trikortikalen Beckenkammspanimplantates ist jedoch mit einer nicht unerheblichen Morbidität assoziiert [1, 2, 18]. Additive, vorwiegend mechanische Probleme des Beckenkammspanimplantats wie Implantatversagen mit kyphotischer Deformität [23] und Implantatwanderungen [13] führten zum zunehmenden Ersatz durch Spongiosa-augmentierte intervertebrale Cages [6, 21, 26, 27, 28]. Mittlerweile befinden sich zahlreiche Cages in klinischer Anwendung. Nach Weiner [36] lassen sich Cages in 3 Designgruppen einteilen: Schraubendesigns, Boxdesigns und Zylinderdesigns.

Intervertebrale Cages wurden mit der Zielsetzung entwickelt, die mechanische Stabilität des Bewegungssegmentes zu gewährleisten [4, 7, 22]. Unter biomechanischen Gesichtspunkten kann die Stabilität des Cage-fixierten Bewegungssegmentes in eine Primärstabilität und eine Sekundärstabilität unterteilt werden [36]. Während die Primärstabilität die direkt postoperativ erzielte Stabilität beschreibt, wird die Sekundärstabilität der Implantate durch deren knöchernen Einheilung erreicht.

Die höhere Primärstabilität unterschiedlicher Cagedesigns im Vergleich zum Beckenkammspanimplantat wurde in zahlreichen biomechanischen In-vitro-Untersuchungen an Hals- und Lendenwirbelsäulenpräparaten dokumentiert [6, 12, 15, 17, 21, 22, 24, 25, 29, 34, 35]. Jedoch existieren nur wenige biomechanische Studien die unterschiedliche Cagedesigns miteinander vergleichen [21, 30]. Zudem ist die aus diesen Untersuchungen resultierende Datenlage nicht konstant. So konnte Oxland [30] z. B. beim Vergleich von Box- und Zylinderdesign-Cages an der Lendenwirbelsäule keinen Unterschied zwischen den Cagedesigns nachweisen. Eigene Untersuchungen [21] an der Halswirbelsäule zeigten jedoch bei ähnlichem Versuchsaufbau eine signifikant höhere mechanische Stabilität von Boxdesign-Cages. Darüber hinaus ist derzeit unbekannt, welcher Zusammenhang zwischen der Primärstabilität eines Cages und dem knöchernen Einheilungsvorgang (Sekundärstabilität) besteht [17]. Vermutet wird, dass eine höhere Primärstabilität durch die „mechanische Ruhe" im Bewegungssegment zu einem besseren Einheilen des Cages und damit zu einer höheren Sekundärstabilität führt [6, 17, 21, 22, 24, 29, 31, 34, 35].

Die Sekundärstabilität eines Cages ergibt sich einerseits aus der Fähigkeit des Implantats, die strukturellen Integrität des Zwischenwirbelraumes zu erhalten [16, 33] und andererseits den biologischen Durchbauungsvorgang zu fördern 5, 17, 36]. Als entscheidender Designparameter für den Erhalt der Zwischenwirbelraumhöhe wurde die Endplattenkonfiguration des Cages definiert [15, 33]. Hierbei wurde die einfache Gleichung formuliert: je größer die Auflagefläche des Cage desto geringer die Sinterung des Cages in vivo [15]. Zusätzlich weisen experimentelle Untersuchungen darauf hin, dass die maximale Pore in der Auflagefläche eines Cages eine entscheidende Funktion für den knöchernen Durchbauungsvorgang aufweist [8, 9, 17]. Kanayama [17] konnte nachweisen, dass eine größere Pore in der Auflagefläche zu einer Reduktion der Stressprotektion der inkorporierten Spongiosa im Cage führt. Des Weiteren vermutete er, dass es durch die Reduktion der Stressprotektion zu einer Stimulation der inkorporierten Spongiosa kommt und somit das Einheilungsverhalten des Cages in vivo verbessert würde [17].

Die oben genannten Anforderungsprofile an das Design eines Cages stehen sich konkurrierend gegenüber. Ein idealer Cage müsste demzufolge sowohl eine maximale Auflagefläche als auch eine maximale Pore in der Auflagefläche aufweisen.

Ziel dieser Untersuchung war es daher, die folgenden Hypothesen in Bezug auf ihre klinische Relevanz zu überprüfen:

1. Je größer die Auflagefläche des Cages, desto geringer die Sinterung des Cages.
2. Je größere die maximale Pore in der Auflagefläche eines Cages, desto geringer das „stress shielding" auf die inkorporierten Spongiosa und desto besser das Einheilungsverhalten des Cages.
3. Je höher die biomechanische Primärstabilität eines Cages, desto höher die biomechanische Sekundärstabilität.

Hierzu wurden aus Voruntersuchungen [21] 2 klinisch etablierte intervertebrale Implantate ausgewählt, die sich sowohl in der Auflagefläche als auch in der maximalen Pore des Cages deutlich unterscheiden. Beide Cages wurden in einem Wirbelsäulenfusionsmodell der Schafshalswirbelsäule [20] in vitro und in vivo untersucht und mit dem trikortikalen Beckenkammspanimplantat als „golden standard" verglichen.

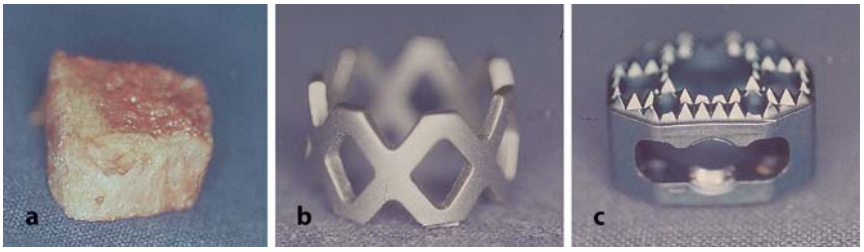


Abb. 1a–c ◀ **Implantate. Bemerkenswert ist der Unterschied in der Auflagefläche und der maximalen Pore der beiden Cagedesigns (s. auch Tabelle 1).** a **Beckenkammspan,** b **Harmscage,** c **Syncage-C**

Material and Methode

Implantate

Die verwendeten Cages sowie der Beckenkammspan sind in Abb. 1 dargestellt. Die wesentlichen Designparameter der Cages wurden in Voruntersuchungen [17, 21] ermittelt und sind in Tabelle 1 dem Beckenkammspanimplantat gegenübergestellt. Der Intra-Cage-Kompressionsdruck beschreibt dabei die Druckkräfte die innerhalb eines Cages wirken. Unter maximaler Pore versteht man die größte Kontinuitätsunterbrechung der Auflagefläche eines Cages. Wesentliche Unterschiede zwischen den Cages bestehen für die Endplatten-Implantat-Kontaktfläche, die maximale Pore und den Intra-Cage-Kompressionsdruck. Während der Harmscage im Vergleich zum Syncage-C eine geringere Auflagefläche aufweist, besitzt er eine größere Pore, die zu einem höheren Intra-Cage-Kompressionsdruck auf die inkorporierte Spongiosa führt (s. Tabelle 1; [17]).

In-vitro-Untersuchungen

Präparate

Es wurden 24 intakte Wirbelsäulenexplantate (C2–C5) von 2 Jahre alten weiblichen Merinoschafen sowie 8 autologe Beckenkammpräparate der Schafe entnommen. Die En-bloc-Präparate wurden bei –20°C gelagert und im Warmwasserbad bei 25°C für die biomechanische Testung aufgetaut. Die Muskulatur wurde entfernt und das Bewegungssegment C3/C4 dargestellt. Besonderes Augenmerk wurde darauf gelegt, alle ligamentären und ossären Strukturen zu erhalten. Jedes Präparat wurde radiologisch untersucht, um sicher zu stellen, dass keine Osteolysen, Frakturen oder andere Abnormalitäten vorlagen.

Um klinische relevante Verhältnisse zu simulieren, wurde eine komplette Diskektomie C3/C4 unter Resektion des vorderen Längsbandes durchgeführt. Die Endplatten wurden mit einer Hochgeschwindigkeitsfräse angefrischt. Alle Cages wurden gemäß Herstellerinformation implantiert. Der Beckenkammspan wurde in der Technik nach Robinson [32] im Intervertebralraum „press-fit“ fixiert.

Biomechanische Testung

Jedes Präparat diente als seine eigene Kontrollgruppe und wurde in der folgenden Sequenz getestet:

1) intaktes Präparat (Kontrollgruppe; n=24). Anschließend wurde eine Diskektomie C3/C4 vorgenommen und sämtliche Präparate randomisiert den jeweiligen Studiengruppen zugeordnet;
2) autologer trikortikaler Beckenkammspan (n=8),
3) Harmscage (n=8) und
4) Syncage-C (n=8).

Das Bewegungssegment C3/C4 wurde mittels nichtdestruktiver Steifigkeitsmessung getestet. Der hierfür verwendete Versuchsaufbau wurde bereits beschrieben [19, 20, 21]. Um die Bewegungen Flexion, Extension, Links- und Rechtsneigung und Links- und Rechtsrotation zu induzieren, wurde ein System aus Seilzügen und Rollen verwendet. Mit einer uniaxialen Materialprüfmaschine (Zwick 1456, Zwick GmbH, Ulm) wurden Kräfte auf das Seilzugsystem ausgeübt. Die aufgebrachten Kräfte konnten mit einer axialen Kraftmessdose (Z12, HBM, Darmstadt), die im Testaufbau integriert ist, gemessen werden. Die reinen Biegemomente wurden durch Multiplikation der aufgewendeten Kräfte mit dem Radius der Rollen am Wirbelsäulenteststand berechnet.

Die dreidimensionalen Bewegungen von C3/C4 wurden mithilfe eines optischen Messsystems (Qualysis Inc., Sävebalden, Schweden) erfasst. Hierzu wurden 2 aus jeweils 3 Messpunkten bestehende Marker (Qualysis Inc., Sävebalden, Schweden) an der Wirbelkörpervorderwand von C3 und C4 ange-

Tabelle 1
Spezifikation der Designparameter der Implantate. (Aus [17, 21]

Implantat	Hersteller	Material	Höhe [mm]	Breite [mm]	Tiefe (mm)	Implantatvolumen [cm^3]	EIKFo [cm^2]	EIKFu [cm^2]	Max. Pore [cm^2]	ICKD [kPa]
BKS	–	Knochen	7	14	14	1,32	–	–	–	–
Harms	DePuyAcroMed	Titan	7	14	14	0,10	0,10	0,10	1,13	314
Syncage-C	Synthes	Titan	7	15	13	0,26	0,26	0,21	0,63	178

EIKF *Endplatten-Implantat-Kontaktfläche;* o *oben;* u *unten;* Max. *Pore maximale Pore des Cages;* ICKD *Intra-Cage-Kompressionsdruck.*

bracht. Die Positionen der Marker wurden mit 2 Kameras erfasst und mit einem computergesteuerten Bewegungsanalysesystem (PC-Reflex, Qualysis Inc., Sävebalden, Schweden) aufgezeichnet. Die anguläre Beweglichkeit von C3 über C4 wurde von speziell angefertigter Computersoftware berechnet. Die experimentelle Unschärfe dieser Methode beträgt ±0,12°.

Beide Wirbelkörper wurden mithilfe von PMMA (Technovit 3040; Heraeus Kulzer GmbH, Wehrheim/Taunus) in Fixationstöpfen eingebettet. Das Gewicht des apikalen Fixationstopfes führte zu einer axialen Vorlast von 25 N, etwa dem Kopfgewicht eines Schafes. Die Präparate wurden während der gesamten Tests feucht gehalten. Zunächst wurden 3 Zyklen à 6 Nm durchgeführt, um eine viskoelastische Relaxation zu ermöglichen. Für den 4. Zyklus (ebenfalls 6 Nm) wurden die Messergebnisse aufgezeichnet. Der Bewegungsumfang, die neutrale und elastische Zone sowie die Steifigkeit wurden anhand der Last-Dislokationskurven bestimmt.

In-vivo-Untersuchungen

Studiendesign

Bei 24 ausgewachsenen (2 Jahre alten) weiblichen Merinoschafen wurde eine ventrale interkorporelle Spondylodese C3/C4 durchgeführt. Die Versuchstiere wurden randomisiert auf die folgenden Gruppen verteilt:

- Gruppe 1: autologer trikortikaler Beckenkammspan (n=8);
- Gruppe 2: Harmscage, gefüllt mit autologer Spongiosa (n=8);
- Gruppe 3: Syncage-C, gefüllt mit autologer Spongiosa (n=8);

Nach 12 Wochen wurden die Schafe getötet und radiologisch, biomechanisch und histologisch evaluiert. Die Untersuchungen wurden durch die lokalen Tierschutzbehörden genehmigt.

Operationstechnik und postoperative Nachsorge

Die Operationstechnik wurde im Detail in vorangegangenen Untersuchungen beschrieben [20]. Über einen linkseitigen anterolateralen Zugang wurde das Bewegungssegment C3/C4 exponiert. Unter Zuhilfenahme eines Kaspar-Distraktors erfolgte die Diskektomie C3/C4 unter Erhalt des Lig. longitudinale posterior. Anschließend wurden die angrenzenden Endplatten mittels einer 2 mm starken Hochgeschwindigkeitsfräse angefrischt, bis petechiale Blutungen auftraten. Intraoperativ wurde das Volumen des autologen Knochenmaterials, entnommen aus dem linken Beckenkamm, nach dem archimedischen Prinzip bestimmt. Schließlich wurden die Harmscages bzw. Syncage-C-Implantate mit jeweils 1,5 cm^3 autologer Beckenkammspongiosa gefüllt und im Intervertebralraum platziert. Der autologe trikortikale Beckenkammspan wurde in der Technik nach Robinson [32] im Intervertebralraum „press-fit“ fixiert. Anschließend wurde die Wunde verschlossen.

Postoperativ wurden die Versuchstiere für 5 Tage mit 0,5 g Metamizol-Natrium (Novaminsulfon®, Lichtenstein) 2-mal täglich intramuskulär analgesiert. Eine klinische Kontrolluntersuchung der Tiere erfolgte in den ersten 10 Tagen täglich, anschließend wöchentlich. Zwölf Wochen postoperativ wurden die Versuchstiere nach Narkotisierung mit einer Überdosis Kaliumchlorid getötet. Anschließend wurde die komplette Halswirbelsäule entnommen.

Radiologische Evaluation

Die Details der radiologischen Evaluation wurden bereits in Voruntersuchungen beschrieben [20]. Prä-, postoperativ und nach 1, 2, 4, 8, 12 Wochen wurden digitale Röntgenbilder (Röntgengerät: Mobilett Plus, Siemens AG, Röntgenbild: Fuji CR 24×30) der Schafshalswirbelsäule in seitlicher und posterioranteriorer Projektion erstellt. Zum selben Zeitpunkt wurden die vordere, mittlere und hintere Bandscheibenraumhöhe und der Intervertebral- und Grunddeckplattenwinkel auf seitlichen Röntgenbildern bestimmt [20]. Die durchschnittliche Bandscheibenraumhöhe wurde aus vorderer, mittlerer und hinterer Bandscheibenraumhöhe errechnet (vordere, mittlere und hintere Bandscheibenraumhöhe/3). Zusätzlich wurde der intervertebrale Höhenindex nach Sandhu [33] bestimmt. Sowohl die durchschnittliche Bandscheibenraumhöhe als auch der intervertebrale Höhenindex wurden zur Bestimmung der Sinterung der Implantate herangezogen. Der Intervertebral- und Grunddeckplattenwinkel wurden vermessen um die Kyphosierung des Bewegungssegmentes zu vermessen. Alle radiologischen Parameter wurden von 3 unabhängigen Untersuchern ausgewertet.

Biomechanische Evaluation

Nach der Tötung der Tiere wurde das Bewegungssegment C3/C4 mittels nichtdestruktiver Steifigkeitsmessung in Analogie zu der In-vitro-Testung biomechanisch evaluiert.

Histologische Evaluation

Im Anschluss an die biomechanische Testung wurde das Halswirbelsäulenpräparat histologisch aufgearbeitet. Hierzu wurde das Bewegungssegment C3/C4 für 7 Tage in gepuffertem Formalin fixiert, in aufsteigender Alkoholreihe entwässert und entfettet und schließlich unentkalkt in Methylmetacrylat (Technovit 9100, Heraeus Kulzer GmbH, Deutschland) eingebettet.

Für die histomorphologische und die histomorphometrische Analyse wurden 6 µm starke longitudinale Schnitte in der Sagittalebene mit einem Leica-SM-2500S-Mikrotom angefertigt. Nach Entfernung der residualen Cage-Bestandteile wurden folgende Färbungen angefertigt: a) Safranin-O/Lichtgrün, b) Safranin-O/v. Kossa, c) Astrablau und d) Masson-Goldner.

Die Masson-Goldner-Präparate wurden für die histomorphologische Analyse verwendet. Die Safranin-O/Lichtgrün-, Safranin-O/v. Kossa- und Astrablau-Präparate wurden für die histomorphometrische Analyse herangezogen. Zur Vermessung histomorphometrischer Parameter wurde eine ROI („region of interest“) definiert, die den gesamten Bandscheibenraum beinhaltete. Die ROI wurde durch die sog. midsagittale Distanz (Distanz zwischen Hinter- und Vorderkante der Deckplatte C4 in der midsagittalen Ebene) und die durchschnittliche präoperative Bandscheibenraumhöhe festgelegt. In dieser ROI wurden mit einem Leica-DM-RB-Mikroskop und unter Zuhilfenahme eines Bildanalysesystems (Zeiss KS 400, Zeiss GmbH, Jena, Deutschland) die folgenden Parameter bei einer

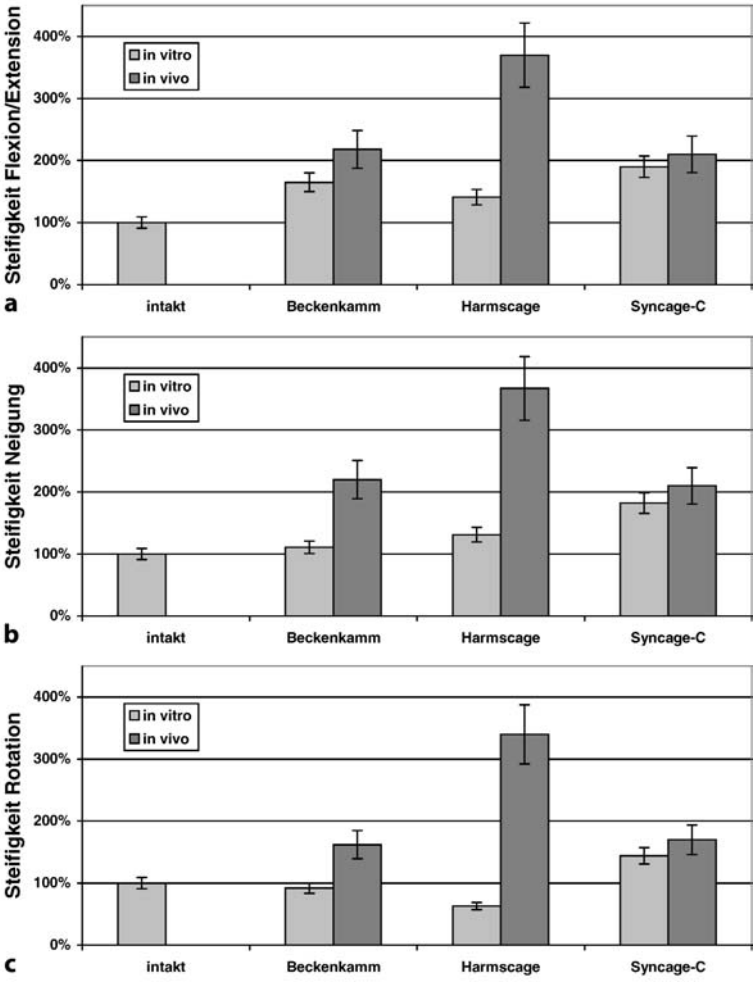


Abb. 2a–c ◀ **Ergebnisse der Steifigkeitsmessung in Relation zum intakten Bewegungssegment (=100%; zur statistischen Auswertung s. Text).** a **In-vitro- und In-vivo-Ergebnisse der biomechanischen Testung in Flexion/Extension.** b **In-vitro- und In-vivo-Ergebnisse der biomechanischen Testung in Neigung.** c **In-vitro- und In-vivo-Ergebnisse der biomechanischen Testung in Rotation**

1,6fachen Vergrößerung vermessen: Knochenvolumen/Gesamtvolumen (KV/GV), Knorpelvolumen/Gesamtvolumen (CV/GV), mineralisiertes Knorpelvolumen/Knorpelvolumen (mCV/CV).

Statistik

Zum Datenvergleich wurde eine One-way-ANOVA für unabhängige Stichproben, gefolgt von einer TUKEY-post-hoc-Analyse für multiple Vergleiche und einer Bonferroni-Korrektur für multiple Messungen durchgeführt. Die Intraobservervariabilität für die radiologischen Messungen wurden mittels Kappa-Statistik ermittelt. Statistisch signifikante Unterschiede wurden bei einen 95%-Konfidenz-Intervall angenommen. Die Werte wurden als Durchschnitt±Standardabweichung angegeben. Das Programm SPSS (Version 10.0, SPSS Inc. Chicago, Illinois) unterstützte die statistische Auswertung.

Ergebnisse

In-vitro-Untersuchungen

Biomechanik

Die biomechanischen In-vitro-Ergebnisse für die Steifigkeit in den Bewegungsrichtungen Flexion/Extension, Rotation und Neigung sind in Abb. 2a–c in Relation zum intakten Bewegungssegment dargestellt.

Im Vergleich zum intakten Bewegungssegment konnten in Flexion/Extension alle Implantate, in Neigung der Harms- und der Syncage-C und in Rotation nur der Syncage-C die Steifigkeit signifikant ($p<0,05$) erhöhen. Verglichen mit dem Beckenkammspanimplantat zeigte der Harmscage eine signifikant ($p<0,05$) höhere Steifigkeit in Neigung, jedoch eine signifikant ($p<0,05$) geringere Steifigkeit in Flexion/Extension und Rotation. Im Gegensatz dazu fand sich für den Syncage-C in allen Bewegungsrichtungen eine signifikant ($p<0,05$) höhere Steifigkeit als für das Beckenkammspanimplantat. Beim Vergleich beider Cages ergab sich für den Syncage-C in allen Bewegungsrichtungen eine signifikant ($p<0,05$) höhere Steifigkeit als für den Harmscage.

In-vivo-Untersuchungen

Radiologische Ergebnisse

Die Intraobservervariabilität für die radiologischen Untersuchungen (Abb. 3) war gut bis sehr gut (Kappa-Werte zwischen 0,76 und 0,92).

Für die präoperativ erhobenen radiologischen Parameter ergab sich kein signifikanter Unterschied zwischen den Gruppen. Nach 8 und 12 Wochen zeigten beide Cage-Gruppen

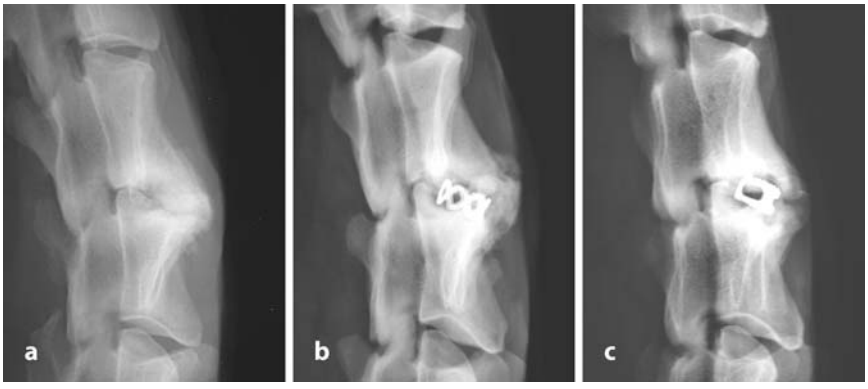


Abb. 3a–c ◀ **Radiologische Ergebnisse: seitliches Röntgenbild der Versuchsgruppen 12 Wochen postoperativ.** a **Gruppe 1: Beckenkammspan,** b **Gruppe 2: Harmscage,** c **Gruppe 3: Syncage-C**

(Gruppe 2 und 3) signifikant ($p<0,05$) höhere Werte für die durchschnittliche Bandscheibenraumhöhe (Abb. 4a) und den intervertebralen Höhenindex als die Beckenkammspangruppe (Gruppe 1). Beim Vergleich beider Cages wies der Syncage-C (Gruppe 3) nach einer Woche signifikant ($p<0,05$) höhere Werte für die durchschnittliche Bandscheibenraumhöhe und den intervertebralen Höhenindex auf als der Harmscage (Gruppe 2). Abgesehen davon konnten für diese beiden Sinterungsparameter keine Unterschiede zwischen beiden Cages evaluiert werden. Nach 2, 4, 8 und 12 Wochen zeigten beide Cages (Gruppe 2 und 3) signifikant ($p<0,05$) höhere Werte für den Intervertebral- und Grunddeckplattenwinkel als das Beckenkammspanimplantat (s. Abb. 4b). Außer zum 2-Wochen-Zeitpunkt, bei dem der Syncage-C (Gruppe 3) signifikant ($p<0,05$) höhere Werte für den Intervertebral- und Grunddeckplattenwinkel aufwies als der Harmscage (Gruppe 2), konnten zwischen den beiden Cages keine Unterschiede für diese beiden Parameter ermittelt werden.

Biomechanische Ergebnisse

Die biomechanischen In-vivo-Ergebnisse für die Steifigkeit in den Bewegungsrichtungen Flexion/Extension, Rotation und Neigung sind in Abb. 2a–c in Relation zum intakten Bewegungssegment dargestellt.

Im Vergleich zum intakten Bewegungssegment konnten alle Implantate in allen Bewegungsrichtungen die Steifigkeit in vivo signifikant ($p<0,05$) erhöhen. Verglichen mit dem Beckenkammspanimplantat (Gruppe 1) zeigte der Harmscage (Gruppe 2) eine signifikant ($p<0,05$) höhere Steifigkeit in allen Bewegungsrichtungen. Im Gegensatz dazu fand sich für den Syncage-C (Gruppe 3) kein signifikanter Unterschied im Vergleich zum Beckenkammspanimplantat (Gruppe 1). Beim Vergleich beider Cages ergab sich für den Harmscage (Gruppe 2) in allen Bewegungsrichtungen eine signifikant ($p<0,05$) höhere Steifigkeit als für den Syncage-C (Gruppe 3).

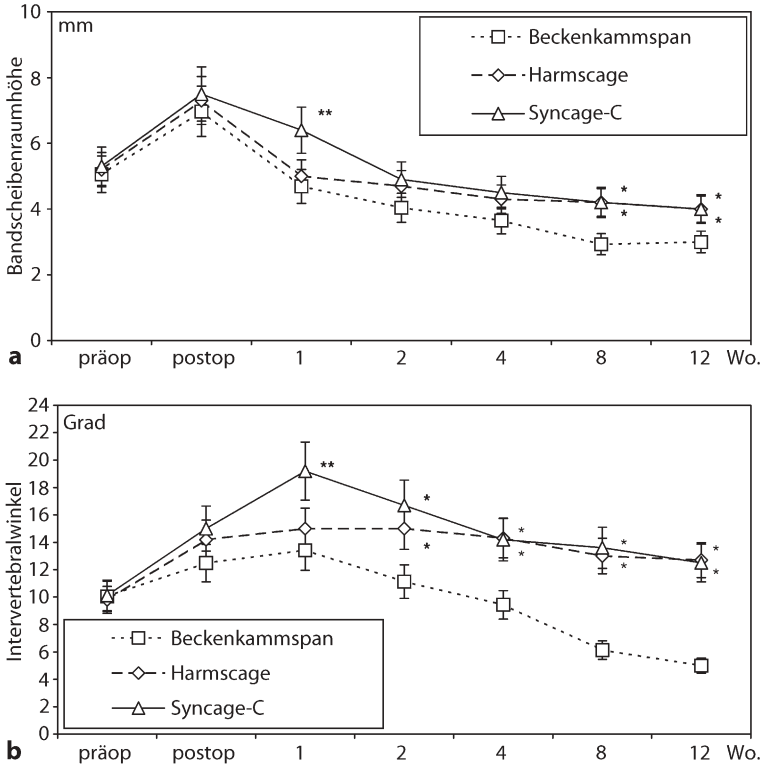


Abb. 4 ▶ a **Radiologische Ergebnisse: durchschnittliche Bandscheibenraumhöhe der verschiedenen Gruppen während des Untersuchungszeitraums (*p<0,05 im Vergleich zum Beckenkammspan, **p<0,05 im Vergleich zum Beckenkammspan und zum Harmscage).** b **Radiologische Ergebnisse: Intervertebralwinkel der verschiedenen Gruppen während des Untersuchungszeitraums (*p<0,05 im Vergleich zum Beckenkammspan, **p<0,05 im Vergleich zum Beckenkammspan und zum Harmscage)**

Abb. 5a–c ▲ **Histomorphologische Ergebnisse: Midsagittaler histologischer Schnitt (Safranin-O/v. Kossa-Färbung) durch den Intervertebralraum 12 Wochen postoperativ (M: 1,6x).** a **Gruppe 1: Beckenkammspan,** b **Gruppe 2: Harmscage,** c **Gruppe 3: Syncage-C**

Beim Vergleich der biomechanischen In-vitro- und In-vivo-Untersuchung innerhalb der Gruppen fand sich für den Harmscage und das Beckenkammspanimplantat eine signifikant ($p<0,05$) höhere Steifigkeit in vivo. Im Gegensatz dazu fand sich für den Syncage-C kein signifikanter Unterschied zwischen In-vivo- und In-vitro-Steifigkeit.

Histomorphologische Ergebnisse

Die histomorphologische Analyse (Abb. 5) unterstützte die Ergebnisse der radiologischen und biomechanischen Untersuchungen (s. Abb. 3).

In der Beckenkammspangruppe (Gruppe 1) fanden sich sowohl zwischen den Endplatten als auch ventral des stabilisierten Bewegungssegmentes Kallusformationen mit teilweise kartilaginären und bindegewebigen Anteilen. Der trikortikale Beckenkammspan war dabei weitgehend kollabiert und durch Osteoklastenaktivität resorbiert. Die Harmscage- und Syncage-C-Gruppe (Gruppe 2 und 3) zeigte zwischen den Endplatten Kallusformationen mit Knocheninseln und Knorpelnestern, die bevorzugt innerhalb der Cages lokalisiert waren. In beiden Gruppen war der Knochen teilweise bis direkt an die Cages angewachsen, partiell waren die Cages aber auch von Bindegewebe umgeben. In der Knochen/Implantat-Grenzfläche zeigten sich keine wesentlichen Unterschiede zwischen beiden Cage-Designs. Speziell in der Syncage-C-Gruppe (Gruppe 3) fand sich jedoch eine ausgedehnte Osteoklastenaktivität, die zu einer weitgehenden Resorption der inkorporierten Spongiosa geführt hatte.

Tabelle 2
Ergebnisse der histomorphometrischen Evaluation 12 Wochen postoperativ. Dargestellt sind die Ergebnisse der midsagittalen Distanz (SD, Basisparameter) sowie des Verhältnis Knochenvolumen/Gesamtvolumen (BV/TV), Knorpelvolumen/Gesamtvolumen (CV/TV) und mineralisiertes Knorpelvolumen/Knorpelvolumen (mCV/CV) im Intervertebralraum (ROI „region of intrest").

Parameter	Gruppe 1 (n=8) Beckenkammspan	Gruppe 2 (n=8) Harmscage	Gruppe 3 (n=8) Syncage-C
SD [mm]	26,2±1,0 (25,0–28,3)	25,5±1,1 (24,6–27,8)	26,1±0,8 (25,2–28,0)
BV/TV [%]	31,4±3,9 (24,8–39,0)	45,5±6,7*, ** (38,5–61,3)	31,6±3,8 (20,5–42,3)
CV/TV [%]	10,1±2,8 (1,0–22,1)	4,6±2,7* (0,8–9,4)	4,2±1,1* (2,8–6,4)
mCV/CV [%]	5,5±2,0 (0,6–9,4)	2,8±1,3* (0,2–7,6)	3,2±1,4* (1,0–5,1)

*$p<0,05$ im Vergleich zur Gruppe 1; **$p<0,05$ im Vergleich zur Gruppe 3.

Histomorphometrische Ergebnisse

Die histomorphometrischen Ergebnisse sind in Tabelle 2 dargestellt. Zwischen den Gruppen konnte kein Unterschied in der sog. midsagittalen Distanz (Basisparameter zur Definition der ROI) festgestellt werden. Im Vergleich zur Beckenkammspangruppe (Gruppe 1) und zur Syncage-C-Gruppe (Gruppe 3) fand sich in der Harmscage-Gruppe (Gruppe 2) eine signifikant ($p<0,05$) fortgeschrittene intervertebrale Knochenmatrixformation (Knochenvolumen/Gesamtvolumen-Verhältnis). Die Beckenkammspangruppe hingegen zeigte für die Parameter Knorpelvolumen/Gesamtvolumen und mineralisiertes Knorpelvolumen/Knorpelvolumen signifikant ($p<0,05$) höhere Werte als die beiden Cage-Gruppen (Gruppe 2 und 3).

Diskussion

Ziel dieser Untersuchung war es, folgende Hypothesen auf ihre Bedeutung zu prüfen:

1. Je größer die Auflagefläche des Cages, desto geringer die Sinterung des Cages.
2. Je größer die maximale Pore in der Auflagefläche eines Cages, desto geringer das „stress shielding" auf die inkorporierten Spongiosa und desto besser das Einheilungsverhalten des Cages.
3. Je höher die biomechanische Primärstabilität eines Cages, desto höher die biomechanische Sekundärstabilität.

Ein Beobachtungszeitraum von 12 Wochen wurde gewählt, da zu diesem Zeitpunkt die Spondylodese weit fortgeschritten, jedoch noch nicht vollständig ist [10, 33]. Daher sind in dieser frühen Phase der Spondylodese besonders gut biomechanische und histologische Unterschiede im Einheilungsverhalten der Implantate zu demonstrieren [10].

Sinterung der Implantate

Zahlreiche klinische Studien konnten bei der intervertebralen Fusion mit autologem trikortikalen Beckenkammspanimplantaten eine signifikante Reduktion der Bandscheibenraumhöhe und eine Kyphosierung des Bewegungssegmentes im postoperativen Verlauf nachweisen [11, 13, 23, 33]. Diese klinischen Beobachtungen konnte auch über einen Zeitraum von 12 Wochen in der vorliegenden tierexperimentellen Untersuchung durch die Bestimmung des intervertebralen Höhenindex und der durchschnittlichen Bandscheibenraumhöhe sowie des Intervertebralwinkels und Deckgrundplattenwinkels bestätigt werden (s. Abb. 4).

Im Gegensatz dazu wurden intervertebrale Cages mit der Zielsetzung entwickelt, die Höhe des Intervertebralraums und die Lordose des Bewegungssegmentes während der knöchernen Fusion zu erhalten [36]. Jedoch liegt bisher zu diesem Themenkomplex nur eine tierexperimentelle In-vivo-Untersuchung vor. Sandhu [33] konnte in einem Wirbelsäulenfusionsmodell am Schaf nachweisen, dass Schraubendesign-Cages in der Lage sind, die postoperativ erzielte Distraktion und Lordose besser zu erhalten als ein autologes trikortikales Beckenkammspanimplantat. Experimentelle Untersuchungen zu den in dieser Studie verwendeten Cagedesigns (Harmscage=Zylinderdesign; Syncage-C=Boxdesign) hinsichtlich deren Sinterungsverhalten wurden bisher nicht durchgeführt. Die Daten dieser Untersuchung zeigen, dass beide Cages und der trikortikale Beckenkammspan in der Lage waren, postoperativ eine Distraktion und Lordosierung des Bewegungssegmentes in gleichem Ausmaß zu erzielen (s. Abb. 4). Jedoch kam es in alle Gruppen während der 12-wöchigen Nachbeobachtungszeit zu einer signifikanten Sinterung der Implantate, die dazu führte, dass die präoperative Bandscheibenraumhöhe deutlich unterschritten wurde. Zusätzlich zeigte sich in allen Gruppen eine Rekyphosierung des Bewegungssegmentes. Während die Reduktion der Bandscheibenraumhöhe und der Verlust der Lordose in beiden Cage-Gruppen Folge eines Einsinken des Cages in die benachbarten Endplatten war, resultierte die Sinterung und Rekyphosierung in der Beckenkammspangruppe aus einem Kollaps des Implantats. Im Vergleich zum Beckenkammspan waren beide Cages jedoch in der Lage, im 12-Wochen-Verlauf die Bandscheibenraumhöhe und die Lordose signifikant besser zu erhalten. Obwohl der Syncage-C zum 2-Wochen-Zeitpunkt eine geringere Sinterung und Rekyphosierung aufwies als der Harmscage, konnten im weiteren zeitlichen Verlauf keine Unterschiede zwischen den Cagedesigns bestimmt werden. Gemäß der initial geäußerten Hypothese – je größer die Auflagefläche des Cages, desto geringer die Sinterung des Cages – hätte jedoch der Syncage-C, dessen Auflagefläche mehr als doppelt so groß ist wie die des Harmscages (s. Tabelle 1), ein geringeres Sinterungsverhalten aufweisen müssen. Daher ist die oben genante Hypothese zu verwerfen. Die Auflagefläche eines Cages scheint die Sinterung des Implantates höchstens in der direkten postoperativen Phase positiv zu beeinflussen, ohne jedoch einen deutlichen Effekt auf den Endzustand (in dieser Studie 12 Wochen) zu haben.

Einheilungsverhalten der Implantate

Zum Einheilungsverhalten der hier untersuchten Cagedesigns liegen bisher keine tierexperimentellen In-vivo-Studien vor. Jedoch wurden Schraubendesign-Cages intensiv in vivo untersucht [3, 10, 14, 37]. So konnten mehrere Autoren ein ausreichendes Einheilen von spongiosagefüllten schraubendesignartigen Cages in zahlreichen Tiermodellen histologisch nachweisen [3, 10, 14, 33, 37].

Die histomorphometrische Analyse in dieser Studie 12 Wochen postoperativ zeigte eine signifikant höhere intervertebrale Knochen-Gesamtvolumen-Relation in der Harmscage-Gruppe als in der Beckenkammspangruppe. Dabei ist die ungünstige Knochen-Gesamtvolumen-Relation des Knochenspanimplantats vorrangig auf dessen mechanisch induzierte Fragmentierung zurückzuführen. Kein Unterschied in der Knochen-Gesamtvolumen-Relation konnte zwischen dem Syncage-C und dem Beckenkammspanimplantat evaluiert werden. Beim Vergleich beider Cage-Gruppen zeigte sich kein Unterschied in der Konfiguration der Knochen/Implantat-Grenzfläche. Der Harmscage wies jedoch eine signifikant höhere intervertebrale Knochen-Gesamtvolumen-Relation auf als der Syncage-C, was einem akzelerierten Einheilungsverhalten des Harmscages entspricht [37].

Cages wurden unter der Vorstellung entwickelt, dass eine mechanische Protektion des inkorporierten autologen Knochenmaterials eine Einheilung des Implantats fördert [7, 22, 24]. Dabei wurde behauptet, dass ausschließlich die mechanische Stabilität und weniger das Design eines Cages von Bedeutung ist [29, 34]. Kanayama [17] konnte jedoch in einer In-vitro-Untersuchung zeigen, dass das Cagedesign einen signifikanten Einfluss auf die Drucke hat, die innerhalb eines Cages auf die inkorporierte Spongiosa wirken. Er demonstrierte, dass die Größe der maximalen Pore eines Cages entscheidend für die Reduktion der Stressprotektion der inkorporierten Spongiosa ist. Dabei formulierte er folgende, auf dem Wolf-Gesetz basierende Hypothese: „Je größere die maximale Pore in der Auflagefläche eines Cages, desto geringer das „stress shielding" auf die inkorporierte Spongiosa und desto besser daher das Einheilungsverhalten des Cages." [17] Diese Hypothese mag die oben genannten Unterschiede in der intervertebralen Knochen-Gesamtvolumen-Relation beider Cages erklären. Der Harmscage besitzt eine ca. doppelt so große maximale Pore und demzufolge einen deutlich höheren Intra-Cage-Kompressionsdruck (s. Tabelle 1). Daher ist das „stress shielding" der inkorporierten Spongiosa im Harmscage deutlich reduziert. Diese Hypothese erklärt auch die histomorphologisch evaluierte höhere Osteoklastenaktivität innerhalb des Syncage-C. Durch die mechanische Stressprotektion der Spongiosa innerhalb des Syncage-C wird die nicht druckbelastete Spongiosa abgebaut. Demzufolge muss konstatiert werden, dass die maximale Pore eines Cages eine entscheidende Rolle für dessen Einheilungsverhalten hat.

Zusammenhang zwischen Primär- und Sekundärstabilität

Die Primärstabilität eines Cage/Spongiosa-Komposit-Implantats resultiert fast ausschließlich aus den mechanischen Eigenschaften des Cages [17, 21]. Erst sekundär, durch die Ausbildung der intervertebralen Knochenmatrix, trägt die inkorporierte Spongiosa zur Gesamtstabilität des Komplexes bei [36]. Die biomechanischen In-vitro-Untersuchungen zeigten die signifikant größte Steifigkeit in allen Bewegungsrichtungen für den Syncage-C. Im Gegensatz dazu wies dar Harmscage nur in Neigung eine höhere In-vitro-Steifigkeit als der Knochenspan auf. Diese Ergebnisse der In-vitro-Testung am Schafsmodell bestä-

tigen vorangegangene biomechanische Untersuchungen, in denen eine höhere Primärstabilität unterschiedlicher Cages im Vergleich zum Beckenkammspanimplantat nachgewiesen werden konnte [6, 12, 15, 17, 21, 22, 24, 25, 29, 34, 35].

Die In-vivo-Ergebnisse dieser Untersuchung stehen jedoch in eindeutigem Gegensatz zu den biomechanischen In-vitro-Ergebnissen. Während der Beckenkammspan und der Harmscage die In-vitro-Steifigkeit in vivo signifikant steigern konnten, bestand zwischen In-vitro- und In-vivo-Steifigkeit des Syncage-C kein signifikanter Unterschied. In vivo zeigte der Harmscage, in vitro der Syncage-C die signifikant größte Steifigkeit in allen Bewegungsrichtungen. Diese Ergebnisse stehen im Gegensatz zu bisherigen Postulaten, nachdem eine hohe Primärstabilität auch zu einer hohen Sekundärstabilität führt [6, 17, 21, 22, 24, 29, 31, 34, 35]. Die deutlichen Unterschiede zwischen den biomechanischen In-vitro- und In-vivo-Ergebnissen dieser Studie weisen vielmehr darauf hin, dass offensichtlich „biologische Qualitäten" eines Cages existieren, die einen entscheidenden Einfluss auf die Sekundärstabilität haben und die durch reine biomechanische In-vitro-Tests bisher nicht zu determinieren sind. Zu fordern bleibt demzufolge eine tierexperimentelle Evaluation neuer Cage-Designs vor deren Markteinführung.

Schlussfolgerung

Obwohl der Syncage-C deutlich bessere biomechanische In-vitro-Eigenschaften als der Harmscage aufweist, ist innerhalb der ersten 12 Wochen postoperativ das frühe Einheilungsverhalten des Harmscage dem des Syncage-C signifikant überlegen. Offensichtlich bestehen „biologische Qualitäten" intervertebraler Implantate, die das Einheilungsverhalten definieren. Die Ergebnisse dieser Studie zeigen, dass die Auflagefläche für das Sinterungsverhalten eines Cages in vivo nur von untergeordneter Bedeutung ist. Hingegen erscheint die Größe der maximale Pore eines spongiosagefüllten Cages eine „biologische Qualität" darzustellen, die eine positive Korrelation zum Einheilungsverhalten des Implantates in vivo aufweist. Da kein direkter Zusammenhang zwischen der Primärstabilität und der Sekundärstabilität von intervertebralen Implantaten in dieser 12-Wochen-Studie bestand, sind biomechanische In-vitro-Untersuchungen nicht dazu geeignet, das frühe Einheilungsverhalten eines Cages zu prognostizieren. Daher bleibt zu fordern, dass eine tierexperimentelle Evaluation neuer Cagedesigns vor deren Markteinführung vorzunehmen ist.

Literatur

1. Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA (1996) Complications of iliac crest bone graft harvesting. Clin Orthop 329:300–309
2. Banwart JC, Asher MA, Hassanein RS (1995) Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. Spine 20:1055–1060
3. Boden SD, Martin GJ Jr, Horton WC, Truss TL, Sandhu HS (1998) Laparoscopic anterior spinal arthrodesis with rh BMP-2 in a titanium interbody threaded cage. J Spinal Disord 11:95–101
4. Brantigan JW, Steffee AD, Geiger JM (1991) A carbon fiber implant to aid interbody lumbar fusion. Mechanical testing. Spine 16:277–282
5. Brantigan JW, Mc Afee PC, Cunningham BW (1994) Interbody lumbar fusion using a carbon fiber implant versus allograft bone. An investigational study in the Spanish goat. Spine 19:1436–1444
6. Brooke NS, Rorke AW, King AT, Gullan RW (1997) Preliminary experience of carbon fibre cage prostheses for treatment of cervical spine disorders. Br J Neurosurg 11:221–227
7. Brodke DS, Dick JC, Kunz DN, McCabe R, Zdeblick TA (1997) Posterior lumbar interbody fusion. A biomechanical comparison, including a new threaded cage. Spine 22:26–31
8. Burger EH, Klein-Nulend J, Veldhuijzen JP (1992) Mechanical stress and osteogenesis in vitro. J Bone Miner Res 7:327–401
9. Carter DR, Wong M (1988) Mechanical stresses and endochondral ossification in the chondroepiphysis. J Orthop Res 6:148–154
10. Cunningham BW, Kanayama M, Parker LM (1999) Osteogenic protein versus autologous interbody arthrodesis in the sheep thoracic spine. A endoscopic study using the Bagby and Kuslich interbody fusion device. Spine 24:509–518
11. Dennis S, Watkins R, Landaker S, Dillin W, Springer D (1989) Comparison of disc space heights after anterior lumbar interbody fusion. Spine 14:876–878
12. Goh JC, Wong HK, Thambyah A, Yu CS (2000) Influence of PLIF cage size on lumbar spine stability. Spine 25:35–39
13. Goulet JA, Senunas LE, Desilva GL, Greenfield ML (1997) Autogenous iliac crest bone graft. Complications and functional assessment. Clin Orthop 339:76–81
14. Hecht BP, Fischgrund JS, Herkowitz HN, Penman L, Toth JM, Shirkhoda A (1999) The use of recombinant human bone morphogenetic protein 2 (rhBMP-2) to promote spinal fusion in a nonhuman primate anterior interbody fusion model. Spine 24:629–636
15. Hollowell JP, Vollmer DG, Wilson CR, Pintar FA, Yoganandan N (1996) Biomechanical analysis of thoracolumbar interbody constructs. How important is the endplate? Spine 21:1032–1036
16. Jost B, Cripton PA, Lund T, Oxland TR, Lippuner K, Jaeger P, Nolte LP (1998) Compressive strength of interbody cages in the lumbar spine: the effect of cage shape, posterior instrumentation and bone density. Eur Spine J 7:132–141
17. Kanayama M, Cunningham BW, Haggerty CJ, Abumi K, Kaneda K, McAfee PC (2000) In vitro biomechanical investigation of the stability and stress-shielding effect of lumbar interbody fusion devices. J Neurosurg (Spine 2) 93:259–265
18. Kandziora F, Mittlmeier T, Kerschbaumer F (1999) Stage related surgery for cervical spine instability in rheumatoid arthritis. Eur Spine J 8:371–381
19. Kandziora F, Kerschbaumer F, Starker M, Mittlmeier T (2000) Biomechanical assessment of the transoral plate fixation for atlantoaxial instability. Spine 25:555–1561
20. Kandziora F, Pflugmacher R, Scholz M, Schnake K, Schröder R, Mittlmeier T (2001) Comparison between sheep and human cervical spines: an anatomic, radiographic, bone mineral density, and biomechanical study. Spine 26:1028–1037
21. Kandziora F, Pflugmacher R, Schäfer J, Duda G, Haas NP, Mittlmeier T (2001) Biomechanical comparison of cervical spine interbody fusion cages. Spine 26:1850–1857
22. Kettler A, Wilke HJ, Dietl R, Krammer M, Lumenta C, Claes L (2000) Stabilising effect of posterior lumbar interbody fusion cages before and after cyclic loading. J Neurosurg 92:87–92
23. Kumar A, Kozak JA, Doherty BJ, Dickson JH (1993) Interspace distraction and graft subsidence after anterior lumbar fusion with femoral strut allograft. Spine 18:2393–2400
24. Lee SW, Lim TH, You JW, An HS (2000) Biomechanical effects of anterior grafting devices on the rotational stability of spinal constructs. J Spinal Disord 13:150–155
25. Lund T, Oxland TR, Jost B, Cripton P, Grassmann S, Etter C, Nolte LP (1998) Interbody cage stabilisation in the lumbar spine: biomechanical evaluation of cage design, posterior instrumentation and bone density. J Bone Joint Surg Br 80:351–359
26. Majd ME, Vadhva M, Holt RT (1999) Anterior cervical reconstruction using titanium cages with anterior plating. Spine 24:1604–1610
27. Matge G (1998) Anterior interbody fusion with the BAK-cage in cervical spondylosis. Acta Neurochir 140:1–8
28. Munoz FLO, de las Heras BG, Lopez VC, Siguero JJA (1998) Comparison of three techniques of anterior fusion in single-level cervical disc herniation. Eur Spine J 7:512–516
29. Nibu K, Panjabi MM, Oxland T, Cholewicki J (1997) Multidirectional stabilising potential of BAK interbody spinal fusion system for anterior surgery. J Spinal Disord 10:357–362
30. Oxland TR, Hoffer Z, Nydegger T, Rathonyi GC, Nolte LP (2000) A comparative biomechanical investigation of anterior lumbar interbody cages: central and bilateral approches. J Bone Joint Surg Am 82:383–393
31. Rapoff AJ, Ghanayem AJ, Zdeblick TA (1997) Biomechanical comparison of posterior lumbar interbody fusion cages. Spine 22:2375–2379
32. Robinson RA (1964) Anterior and posterior cervical spine fusions. Clin Orthop 35:34–36
33. Sandhu HS, Turner S, Kabo Met al. (1996) Distractive properties of threaded interbody fusion device. An in vivo model. Spine 21:1201–1210
34. Tencer AF, Hampton D, Eddy S (1995) Biomechanical properties of threaded inserts for lumbar interbody spinal fusion. Spine 20:2408–2414
35. Volkman T, Horton WC, Hutton WC (1996) Transfacet screws with lumbar interbody reconstruction: biomechanical study of motion segment stiffness. J Spinal Disord 9:425–432
36. Weiner BK, Fraser RD (1998) Spine update lumbar interbody fusion cages. Spine 23:634–640
37. Zdeblick TS, Ganayem AJ, Rapoff AJ, Swain C, Bassett T, Cooke ME, Markel M (1998) Cervical interbody fusion cages. An animal model with and without bone morphogenetic protein. Spine 23:758–765

Diese Untersuchung konnte zeigen, dass designspezifische Charakteristika von Cages existieren, die das Einheilungsverhalten der Implantate definieren. Von besonderer Bedeutung für das Einheilungsverhalten eines Cages ist das "stress shielding" des Implantates auf die inkorporierte Spongiosa. Der Harmscage weist aufgrund des geringeren "stress shielding" ein signifikant besseres Einheilungsverhalten auf als der Syncage-C. Daher wurde in allen folgenden Untersuchungen der Harmscage eingesetzt.

2.3 Wachstumsfaktoren und Carrier-Systeme

2.3.1 Kollagen- und PDLLA-Carrier zur BMP-2 Applikation

Da bei der Verwendung von Polylaktid-Carriern inflammatorische Reaktionen mit begleitender Ausbildung von Osteolysen beschrieben wurden [53], fanden in der experimentellen Wirbelsäulenchirurgie bisher, trotz zahlreicher Nachteile, vorwiegend Kollagen-Carrier zur lokalen Applikation von Wachstumsfaktoren Anwendung. [127,167,180,184]. Eine neu entwickelte dünne PDLLA-Beschichtung von Implantaten zur lokalen Freisetzung von Wachstumsfaktoren könnte im Vergleich zu derzeit verfügbaren Polylaktid- und Kollagen-Carriern Vorteile aufweisen [73,171].

- Daher sollten in dieser Untersuchung der PDLLA-Carrier hinsichtlich seiner lokalen Wirkung auf die intervertebrale Spondylodese im zervikalen Schafsmodell untersucht werden.
- Zusätzlich sollte die Effektivität des PDLLA-Carriers mit der eines Kollagen-Carriers bei der lokalen Applikation von BMP-2 im Spondylodesemodell der zervikalen Schafswirbelsäule verglichen werden.

J Neurosurg (Spine 1) 97:40–48, 2002

Bone morphogenetic protein–2 application by a poly(D,L-lactide)–coated interbody cage: in vivo results of a new carrier for growth factors

FRANK KANDZIORA, M.D., HERMANN BAIL, M.D., GERHARD SCHMIDMAIER, M.D., GEORG SCHOLLMEIER, M.D., MATTI SCHOLZ, CHRISTIAN KNISPEL, TIMO HILLER, ROBERT PFLUGMACHER, M.D., THOMAS MITTLMEIER, M.D., MICHAEL RASCHKE, M.D., AND NORBERT P. HAAS, M.D.

Unfall- und Wiederherstellungschirurgie, Universitätsklinikum Charité der Humboldt Universität Berlin, Campus Virchow-Klinikum, Berlin, Germany

Object. Growth factors such as bone morphogenetic protein–2 (BMP-2) have been proven to promote spine fusion and to overcome the disadvantages of an autologous bone graft. The optimum method to deliver such growth factors remains a matter of discussion. The purpose of this study was to determine the safety and efficacy of a new poly(D,L-lactide) (PDLLA) carrier system for BMP-2 and to compare this carrier system with a collagen sponge carrier in a sheep cervical spine interbody fusion model.

Methods. Thirty-two sheep underwent C3–4 discectomy and fusion: Group 1, titanium cage (eight animals); Group 2, titanium cage coated with a PDLLA carrier (eight animals); Group 3, titanium cage coated with a PDLLA carrier including BMP-2 (150 μg) (eight animals); and Group 4, titanium cage combined with a collagen sponge carrier including BMP-2 (150 μg) (eight animals). Blood samples, body weight, and temperature were assessed. Radiographs were obtained pre- and postoperatively and after 1, 2, 4, 8, and 12 weeks. At the same time points, disc space height, intervertebral angle, and lordosis angle were measured. After the sheep were killed 12 weeks postoperatively, flexion–extension radiography was performed to evaluate fusion sites. Quantitative computerized tomography scans were obtained to assess bone mineral density (BMD), bone mineral content (BMC), and bone callus volume (BCV). Biomechanical testing was performed in flexion, extension, axial rotation, and lateral bending. Stiffness, range of motion, neutral, and elastic zone were determined. Histomorphological and -morphometrical analyses were performed, and polychrome sequential labeling was used to determine the timeframe of new bone formation.

There were no differences among the groups concerning blood counts, body weight, and temperature. Compared with the noncoated cages, all PDLLA-coated cages showed significantly higher values for BMD of the callus, as well as slightly higher values for BMC, BCV, and the bone volume/total volume ratio. In comparison with the cage-alone group, the BMP-2 groups showed significantly higher values for BMD and biomechanical stiffness. Histomorphological, -morphometrical, and polychrome sequential labeling analyses demonstrated greater progression of callus formation in the BMP-2 groups than in any other group. Compared with BMP-2 delivered using a collagen sponge carrier, BMP-2 application with a PDLLA carrier resulted in a higher BCV and a greater progression of interbody callus formation in the histomorphometrical analysis.

Conclusions. The use of cervical spine interbody fusion cages coated with PDLLA as a delivery system for growth factors was effective. In this 12-week follow-up study, the PDLLA coating showed no adverse effects. The slight but not significant positive effect of the PDLLA carrier on interbody fusion might be a result of the degradation process of the biodegradable carrier. Compared with collagen sponge delivery of BMP-2, the PDLLA-coated interbody cages significantly increased the results of interbody bone matrix formation. In this new combination (implant + PDLLA + growth factor) the cage represents a "real fusion" cage, because it not only serves as a mechanical device for spinal fixation but also as a local drug delivery system.

KEY WORDS • cervical spine • bone morphogenetic protein • interbody fusion • poly(D,L-lactide) • growth factor • sheep

GROWTH factors such as BMP-2 have been proven to accelerate spinal fusion and to overcome the disadvantages of an autologous bone graft.[2–4,6,8,9,13,19,31,36,44] The main disadvantage of BMP-2 is its quick inactivation in vivo after 20 to 30 minutes.[18] Furthermore, because BMP-2 is not specific for particular tissue, it has various effects on cellular processes. Additionally, BMP-2 has dose-dependent effects and may be efficacious at high or low doses.[8,10,42,43] Therefore, the local, controlled, and continuous application of these factors seems to be necessary to accelerate spinal fusion. The optimum delivery method of growth factors such as BMP-2 remains a matter of debate. Currently, collagen-impregnated sponge carriers are used for local application of BMP-2 in experimental spinal fusion.[3,4,44] The safety and accuracy of these carriers, however, are questionable.[30,39,40]

Abbreviations used in this paper: BCV = bone callus volume; BMC = bone mineral content; BMD = bone mineral density; BMP-2 = bone morphogenetic protein–2; CT = computerized tomography; DSH = disc space height; EZ = elastic zone; IVA = intervertebral angle; LA = lordotic angle; NZ = neutral zone; PDLLA = poly(D,L-lactide); PGA = poly(glycolide acid); PLA = poly(lactic acid); rhBMP = recombinant human BMP; ROI = region of interest; ROM = range of motion.

Coating of implants with locally active growth factors could influence concepts of spinal fusion. The continuous local application of these factors at the required site in the desired concentrations, however, remains an unsolved problem. A continuous drug release with high local but low systemic concentrations could be achieved using a biodegradable drug carrier. Recently, investigators have described a new biodegradable local drug delivery system that enables the "cold coating" of implants with potential thermolabile proteins such as BMP-2.[14,37,38] The authors of studies on rat tibia fractures have reported that this PDLLA carrier itself applied via an intramedullary titanium implant had a significantly positive effect on bone matrix formation. Compared with the noncoated implant, the PDLLA-coated implant showed a significantly increased fracture consolidation rate radiographically, biomechanically, and histomorphometrically.[37]

Based on these studies, this new coating technique could be very promising in the field of spinal surgery because we may be able to combine biomechanically well-established implants with biologically active substances. In this new combination the implant serves as a device for spinal fixation as well as a local drug delivery system to improve spinal fusion not only by the growth factors but potentially by the carrier system itself.

The purpose of this study was to determine the safety and efficacy of a biodegradable PDLLA carrier system for BMP-2 and to compare this delivery system with a collagen sponge carrier in a sheep cervical spine interbody fusion model.

Materials and Methods

Study Design

Thirty-two adult (2-year-old) female merino sheep underwent C3–4 discectomy and fusion. Eight sheep were randomly assigned to each of the following groups: Group 1, mesh titanium cage (eight sheep); Group 2, mesh titanium cage coated with biodegradable PDLLA carrier (eight sheep); Group 3, mesh titanium cage coated with biodegradable PDLLA carrier including BMP-2 (150 μg) (eight sheep); and Group 4, mesh titanium cage combined with collagen sponge carrier including BMP-2 (150 μg).

After 12 weeks all sheep were killed, and radiographic, biomechanical, and histological evaluations were performed. All animal experimental work was approved by local authorities.

Cage Preparation

The PDLLA was chosen as the drug carrier system for sheep in Groups 3 and 4. The coating properties and the coating technique have been previously described.[37] In Group 3 rhBMP-2 (5% wt/wt) was incorporated in the PDLLA solution. The mean PDLLA coating mass of the cages was 3.02 ± 0.12 mg. Therefore, approximately 150 μg (5% wt/wt) BMP-2 was incorporated in the coating of each cage.

Preparation and application of BMP-2 and the collagen sponge have been previously described.[36] In Group 4, 1 cm^3 of solution containing 150 μg of rhBMP-2 was added to an established collagen sponge carrier[44] by drip application.

Surgical Technique and Postoperative Care

All sheep received 2 g amoxicillin intravenously before surgery. The animals underwent surgery after induction of general endotracheal anesthesia (0.5 g thiopental-natrium and 0.1 mg fentanyl dihydrogen citrate). For maintenance of anesthesia, inhalation of isoflurane and intravenous 0.2-mg dosages of fentanyl dihydrogen citrate were applied. The anterior part of the neck was prepared in a sterile fashion, and a left anterolateral approach to the cervical spine was performed via a longitudinal skin incision. The longus colli muscle was incised at the midline, and the intervertebral C3–4 disc was exposed. After using a Caspar distractor to distract the motion segment, an anterior discectomy was performed at C3–4. Using a 2-mm high-speed diamond drill, the endplates were shaved down to bleeding bone. No attempt was made to excise the posterior longitudinal ligament or expose the spinal canal. For interbody stabilization, mesh titanium 14-mm-diameter cages of appropriate height (mean height 8 mm) were used. Finally, the wound was irrigated with saline, and the longus colli muscle was closed using a running suture. The subcutaneous tissue and the skin were reapproximated using interrupted sutures, and a soft bandage was applied to the neck.

Postoperatively, the sheep were observed until fully recovered from general anesthesia. They received two 0.5-g doses of metamizol-natrium per day for 5 days intramuscularly. Clinical examination was performed daily for the first 10 days and weekly thereafter. The sheep were allowed ad libitum activity for the remainder of the experimental period. Fluorochrome sequential labels were administered at 3, 6, and 9 weeks postoperatively; these consisted of intravenous administration of the following: oxytetracyclin (25 mg/kg) at 3 weeks, calcein green (15 mg/kg) at 6 weeks, and xylenol orange (90 mg/kg) at 9 weeks. Twelve weeks after surgery, the animals were killed, after induction of 0.5 g thiopental-natrium and 0.1 mg fentanyl dihydrogen citrate, by an intravenous injection of potassium chloride. The complete cervical spine, including parts of the occiput and T-1, was excised and cleaned from the surrounding tissue.

Blood and Serum Analyses

Blood and serum samples were taken from the saphenous vein of the hind leg pre- and postoperatively and after 1, 2, 4, 8, and 12 weeks. The blood samples were analyzed for routine laboratory parameters (blood count, electrolytes, alkaline phosphatase, thyroid values, and glucose).

Body Weight and Temperature

Preoperatively and at 1, 2, 4, 8, and 12 weeks postoperatively rectal body temperature and body weight were measured.

Radiographic Analysis

Lateral and anteroposterior digital radiographs were obtained pre- and postoperatively and at 1, 2, 4, 8, and 12 weeks. At the same time periods, anterior, middle, and posterior intervertebral DSH, IVA, and LA were measured on lateral radiographs.[22] The mean intervertebral DSH was calculated from anterior, middle, and posterior DSH measurements (anterior, middle, and posterior DSH/3). All radiographic measurements were evaluated by three independent observers.

Functional Radiographic Analysis

After the sheep were killed lateral digital functional radiographs (in flexion and extension) were obtained to evaluate the fusion sites. For this purpose, the T-1 segment was rigidly fixed using a Steinmann pin while a 60-N load was applied through C-1 by using a dynamometer. Flexion and extension differences in the IVA and LA were calculated. All functional radiographic measurements were evaluated by three independent observers.

Quantitative CT Analysis

After the sheep were killed, quantitative CT scans were obtained. Axial cuts with 1-mm slice thickness were made parallel to the intervertebral disc space. The BMD measurements were calibrated using a six-point BMD phantom.[22] Measurements were performed using specific software of the scanner. The BCV was measured using a Zeiss image analysis system. The BMC was calculated from BMD and BCV measurements (BMC = BCV × BMD). All CT measurements were evaluated by three independent observers.

Biomechanical Analysis

After the sheep were killed, biomechanical testing was performed

using a nondestructive flexibility method in which a nonconstrained testing apparatus, previously described in detail, was applied.[20,22] Pure bending moments of 6-Nm load were applied to each C3–4 motion segment by using a system of cables and pulleys to induce flexion, extension, left and right lateral bending, and left and right axial rotation. A uniaxial testing machine was used to apply tension. Three-dimensional displacement of each motion segment was measured using an optical measurement system. Triangular markers with three diodes were attached to the bodies of C-3 and C-4. Marker positions were detected using two cameras and recorded with a computerized motion analysis system. Angular displacement of the upper vertebra (C-3) in relation to the lower vertebra (C-4) was calculated from marker position by using custom-made computer software. The experimental error associated with this method was $\pm$ 0.1°.[23] The mean apparent stiffness values in the EZ were calculated from the corresponding load-displacement curves. The ROM, NZ, and EZ were determined.

Histomorphological, Histomorphometrical, and Fluorochrome Analysis

For histological examination of bone, all C3–4 motion segments were harvested after 12 weeks. The motion segments were fixed for 7 days in 10% normal buffered formaldehyde, dehydrated in ascending concentrations of ethanol, and embedded in methylmethacrylate without being decalcified.

For histomorphological and histomorphometrical analyses, longitudinal sections in the sagittal plane were cut at 6 μm by using a microtome and a 40° stainless-steel knife. The residual parts of the cages were then removed, and the following stains were used: Safranin-O/lightgreen, Safranin-O/van Kossa, astrablue, and Masson–Goldner. Masson–Goldner staining was used for histomorphological analysis.

Histomorphometrical parameters were measured on the residual stainings using a Leica microscope and an image analyzing system. Parameters were measured at a magnification of 1.6.

The sagittal diameter distance of C-3 and the mean preoperative DSH were determined to define the size of the ROI for histomorphometrical evaluation. The complete intervertebral fusion area was included in this ROI. The following structural indices were calculated in the ROI: bone volume/total volume, cartilage volume/total volume, and mineralized cartilage volume/cartilage volume.

For fluorochrome analysis, longitudinal sections in the parasagittal plane were cut at 400 μm by using a precise macrogrinding machine. These slices were then ground to a thickness of 80 μm by using a precise microgrinding machine. Fluorochrome markers were analyzed under appropriate lighting conditions by using a Leica microscope and a Zeiss image analysing system. Parameters were measured at a magnification of 1.6.

Fluorochrome analysis of the intervertebral fusion areas has been previously described in detail.[44] The first appearance of the marker served to indicate formation of new bone matrix. The presence or absence of each marker around or within the cage or the bone graft, respectively, was used to determine the relative time frame of new bone formation.

Sources of Supplies and Equipment

We obtained the PDLLA from Boeringer (Ingelheim, Germany). The rhBMP-2 was acquired from the Theodor-Boveri-Institut (Würzburg, Germany). The collagen sponge carrier (Helistat) was produced by Integra Life Sciences (Plainsboro, NJ). The methylmethacrylate (Technovit 9100) was acquired from Heraeus Kulzer GmbH (Wehrheim/Ts, Germany).

The titanium mesh cages were purchased from Motech GmbH (Schwenningen, Germany). Our x-ray unit (Mobilett Plus) was manufactured by Siemens AG (Erlangen, Germany), and the x-ray film (CR 24×30) was produced by Fuji (Kleve, Germany). The dynamometer used to deliver the 60-N load to C-1 was obtained from Inha GmbH (Berlin, Germany). The CT scans were acquired using a scanner (Somatom plus 4) that was manufactured by Siemens, which also produced the specific scanner software (Sienet Magic View VA 30A) used for determining radiographic measurements. An analyzing system (KS 400), used to calculate BCV, was obtained from Zeiss GmbH (Oberkochen, Germany). The uniaxial testing machine (model 1456) was purchased from Zwick GmbH (Ulm, Germany). Qualysis Inc. (Sävebalden, Sweden) produced the optical analysis system, diodes, and computerized motion analysis system (PC-Reflex). We obtained the macro- and microgrinding machines from Fa. Exact (Norderstedt, Germany). The SPSS software was obtained from SPSS (Chicago, IL).

Statistical Analysis

Comparison of data was performed using one-way analysis of variance for independent samples followed by Tukey post-hoc analysis for multiple comparison procedures in which Bonferroni correction for multiple measurements was used. Intraobserver variability for radiographic and functional radiographic evaluation was determined using κ statistics. Statistically significant differences were defined at a 95% confidence level. The values are given as means $\pm$ standard deviations. The SPSS software supported statistical evaluation.

Results

Failure Parameters and Complications

One sheep died of an anesthesia-related complication on Day 0. In Group 1 one animal had to be killed during Week 6 because it developed sore mouth (contagious ecthyma). Both sheep were excluded from the study and replaced by two others. In Group 1 one animal developed a superficial seroma in the neck, which healed without further difficulties after provision of conservative treatment.

Blood and Serum Analysis Results

In an analysis of the full blood count no significant changes among the groups and throughout the experiment were demonstrated. Even after 1 week no significant changes were found for mean hemoglobin, erythrocyte, and leukocyte levels. Levels of electrolytes (Na, K, Cl, and Ca) did not show significant changes during the experimental period. Furthermore no changes of thyroid hormones, alkaline phosphatase, and glucose levels were found throughout the observation period and among all groups.

Body Weight and Temperature

No significant intergroup differences were found in mean body temperature and body weight throughout the experimental period. Postoperatively, a slight and constant increase in body weight for all sheep was determined.

Radiographic Results

Intraobserver agreement for radiographic measurements was good, showing κ values ranging from 0.76 to 0.93. Preoperative baseline radiographic values did not show any differences among the groups. No significant intergroup differences were found for mean DSH, IVA, and LA throughout the observation period.

Functional Radiographic Results

Intraobserver agreement for functional radiographic measurements was excellent (κ values 0.87–0.94). Functional radiographic assessment (Table 1) revealed significantly lower residual flexion–extension movement in the BMP-2-treated groups (Group 3 and 4) than in both other groups ($p < 0.05$). There were no differences between sheep that received coated (Group 2) and noncoated cages

TABLE 1

Summary of functional radiographic data obtained after 12 weeks

Flexion/ Extension	Difference in Degrees (range)			
	Group 1	Group 2	Group 3	Group 4
IVA	8.6 ± 2.6 (6–10)	8.7 ± 2.4 (7–10.5)	2.7 ± 2.1*† (0–7)	3.6 ± 1.8*† (1.5–7)
LA	8.6 ± 3.1 (6–12)	7.9 ± 2.7 (4–9.5)	3.9 ± 2.3*† (0–9)	4.4 ± 1.7*† (2–6.5)

* $p < 0.05$ compared with Group 1.
† $p < 0.05$ compared with Group 2.

(Group 1). Although flexion–extension differences were constantly higher in the collagen sponge–delivered BMP-2 group (Group 4), no significant differences could be detected between the PDLLA-delivered BMP-2 (Group 3) and Group 4 sheep.

Quantitative CT Results

Intraobserver agreement related to CT measurements was excellent (κ values 0.86–0.95). After 12 weeks there were no significant differences in BMC and BCV between the cage-alone group (Group 1) and the cage plus PDLLA group (Group 2). In the PDLLA-coated cage group (Group 2), and in both BMP-2-treated groups (Groups 3 and 4) BMD of the callus was significantly higher than in Group 1 ($p < 0.05$). The PDLLA-coated cages with BMP-2 (Group 3) showed significantly higher values for BCV ($p < 0.05$) than in any other group (Fig. 1 and Table 2). No ossifications of the posterior longitudinal ligament were observed in any group.

Biomechanical Results

The mean stiffness in axial rotation and lateral bending was significantly higher ($p < 0.05$) in the BMP-2–treated sheep (Groups 3 and 4) than in the other two groups (Fig. 2). Additionally, ROM and EZ in rotation and lateral bending were significantly lower ($p < 0.05$) in Groups 3 and 4 than in the other two groups (Table 3). The highest stiffness values and the lowest ROM, NZ, and EZ values were constantly demonstrated in Group 3. No significant difference, however, was determined between Groups 3 and 4.

Histomorphological Results

Histomorphological analysis supported the findings of radiographic and biomechanical examinations (Fig. 3). In the noncoated (Group 1) and PDLLA-coated (Group 2) cage groups, mainly fibroblasts and cartilage cells were observed between the endplates without major differences between the groups. Compared with Group 1, however, small bony islands could be detected surrounding the cages in Group 2. The spines in Groups 3 and 4, stabilized with BMP-2-treated cages, showed advanced remodeled callus with low cartilage and fibrous tissue components; there were no major differences between these groups. Compared with the collagen sponge carrier (Group 4), however, a greater number of bony islands could be detected surrounding the PDLLA-coated cages (Group 3). No differences in the quantity of macrophages, which might indicate degradation products of the carriers, were observed on the histological sections obtained in the different groups. No voids in the interbody fusion mass or inflammatory reaction associated with the PDLLA coating were observed.

Histomorphometrical Results

The results of histomorphometrical analysis are presented in Table 4. No significant differences in sagittal diameter index (baseline) were demonstrated among the groups. Compared with Groups 1, 2, and 4 significantly advanced interbody bone matrix formation was found in Group 3 ($p < 0.05$). There was no significant difference in the bone volume/total volume ratio between the PDLLA-coated cages (Group 2) and the collagen sponge– and BMP-2-treated cages (Group 4). There were no differences in histomorphometrical parameters between Groups 1 and 2 except for bone formation ($p < 0.05$), which was higher in the PDLLA-coated cages.

Fluorochrome Analysis Results

The results of fluorochrome analysis are summarized in Table 5. The BMP-2-coated cages exhibited earlier new bone formation both within and around the cages com-

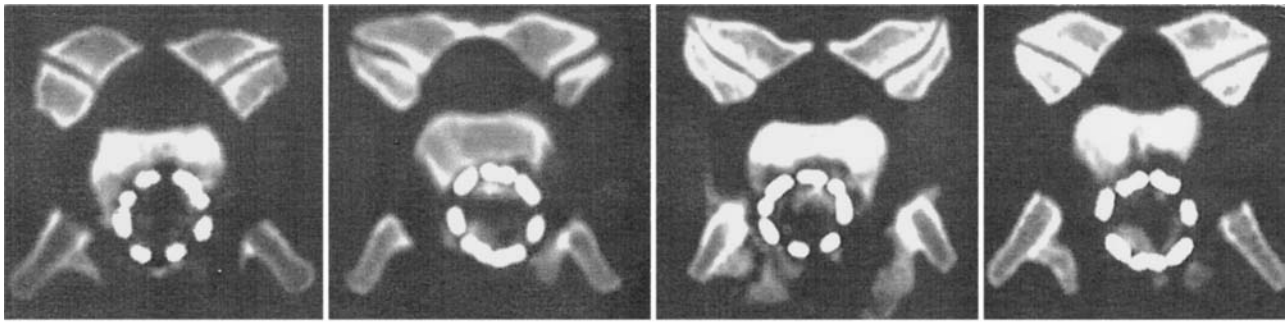


FIG. 1. Representative CT scan obtained after 12 weeks demonstrating interbody fusion parallel to the intervertebral space.

TABLE 2

Summary of quantitative CT data obtained after 12 weeks

Quantitative Scanning	Group 1	Group 2	Group 3	Group 4
BMD (g/cm^3)	0.58 ± 0.04 (0.56–0.65)	0.62 ± 0.03* (0.59–0.66)	0.62 ± 0.04* (0.58–0.65)	0.61± 0.03* (0.58–0.64)
BMC (g)	1.9 ± 0.7 (1.2–2.8)	2.1 ± 0.5 (1.3–2.6)	3.3 ± 1.1* (2–5.5)	2.5 ± 1.0 (2–5.5)
BCV (cm^3)	3.3 ± 1.2 (2–4.7)	3.4 ± 0.9 (2.5–4.3)	5.4 ± 1.9*†‡ (2.1–7.4)	4.1 ± 1.0 (2–5.3)

* $p < 0.05$ compared with Group 1.
† $p < 0.05$ compared with Group 2.
‡ $p < 0.05$ compared with Group 4.

pared with the non–BMP-2-coated other groups. There were no significant differences between Groups 1 and 2 except for new bone formation around the cages at 9 weeks postoperatively, which was greater in the PDLLA-coated cages. No differences throughout the observation period were detected between Groups 3 and 4.

Discussion

The objectives of this study were: 1) to determine the safety and efficacy of a biodegradable PDLLA carrier system for BMP-2 and 2) to compare this carrier system with a collagen sponge carrier in an in vivo sheep cervical spine interbody fusion model using BMP-2.

Biodegradable PDLLA Carrier System

The optimum method for delivering growth factors to the corresponding field of interest remains a matter of debate. For local application of growth factors in experimental spinal fusion, collagen sponge carriers are currently the most popular. The safety and accuracy of these carriers, however, are questionable.[30,39,40] A fast and uncontrolled release of drugs from collagen sponges with potential undesired drug- or carrier system–induced tissue reactions has been described.[39] An accurate intraoperative placement of these carriers at the desired region appears difficult, and inaccurate positioning may lead to uncontrolled interactions with the surrounding tissue or growth factor inefficacy.[1,16,24,33] Martin, et al.,[30] for example, demonstrated in an intertransverse spinal fusion model that soft-tissue compression of the collagen sponge carrier prevented bone induction at standard growth factor doses. Boden, et al.,[3] reported that a resorbable collagen sponge carrier was a suitable vehicle in rabbits and dogs; however, it was found to be subjectable to compression in a lumbar intertransverse spinal fusion model in monkeys. Finally, the safety of the collagen sponge carrier is questionable because it consists partly of bovine material that can cause allergic reactions or infections.[40]

Different techniques for the coating of biomaterials and local drug release have been described. Both PLA and PGA or their copolymers have been widely used as biodegradable implants and drug delivery systems in orthopedic surgery.[8,9,28,35,36] The vehicle examined in our study (PDLLA) undergoes similar degradation to PGA and PLA by chemical, thermal, mechanical, and physical mechanisms,[12] whereas the chemical degradation primarily through hydrolysis and metabolism in the citric acid cycle is the most important.[25,26] Many investigators have studied the biocompatibility of poly(α-hydroxy acids) and the local tissue response of PLA and PGA or their copolymers. The tissue response depends on the amount and the

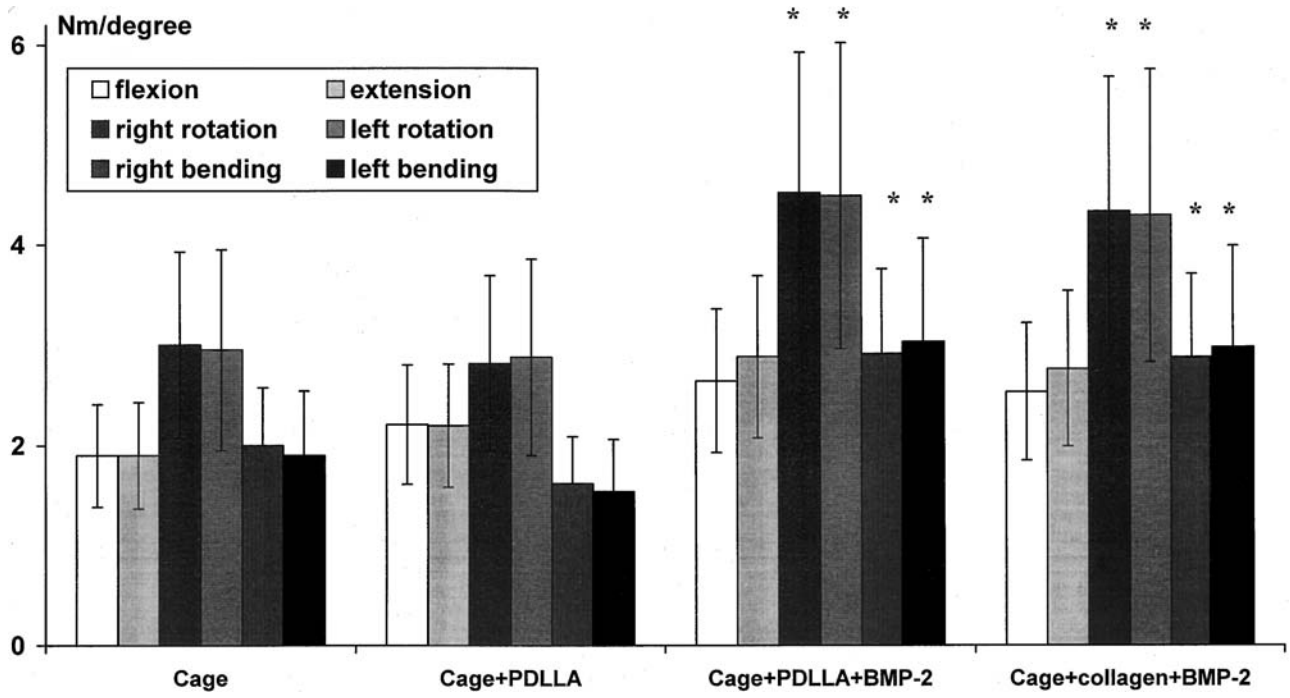


FIG. 2. Bar graph demonstrating results of biomechanical stiffness of the different groups in the different test modes.
* $p < 0.05$ compared with the cage-alone (Group 1) and the cage + PDLLA (Group 2) specimens.

TABLE 3

Summary of biomechanical data obtained after 12 weeks

Motion	Differences in Degrees			
	Group 1	Group 2	Group 3	Group 4
flexion				
ROM	3.9 ± 0.8	3.0 ± 0.9	2.9 ± 1.2	2.8 ± 1.0
NZ	1.3 ± 0.6	0.5 ± 0.7	0.9 ± 1.1	0.9 ± 1.0
EZ	2.6 ± 0.8	2.5 ± 0.9	2.0 ± 0.7	1.9 ± 0.7
Extension				
ROM	3.9 ± 1.3	3.3 ± 1.2	2.7 ± 0.7*	2.9 ± 0.9*
NZ	1.0 ± 0.6	1.0 ± 1.0	0.6 ± 0.5	0.8 ± 0.6
EZ	2.9 ± 0.9	2.3 ± 0.3	2.1 ± 0.3*	2.1 ± 0.5*
rt rotation				
ROM	2.3 ± 1.0	2.4 ± 0.8	1.0 ± 0.3*†	1.2 ± 0.3*†
NZ	0.5 ± 0.1	0.4 ± 0.3	0.2 ± 0.1*†	0.3 ± 0.2*
EZ	2.0 ± 0.9	2.0 ± 0.6	0.8 ± 0.3*†	0.9 ± 0.3*†
lt rotation				
ROM	2.3 ± 0.8	2.3 ± 0.5	1.1 ± 0.4*†	1.3 ± 0.4*†
NZ	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1*†	0.3 ± 0.3*
EZ	1.9 ± 0.7	2.0 ± 0.5	0.9 ± 0.4*†	1.0 ± 0.5*†
rt bending				
ROM	3.8 ± 1.0	4.1 ± 1.0	2.1 ± 0.7*†	2.3 ± 0.9*†
NZ	1.0 ± 0.4	0.7 ± 0.4	0.6 ± 0.2*†	0.6 ± 0.4*
EZ	2.8 ± 0.7	3.4 ± 0.9	1.5 ± 0.7*†	1.7 ± 0.7*†
lt bending				
ROM	3.9 ± 1.2	4.2 ± 0.7	2.1 ± 0.9*†	2.4 ± 1.1*†
NZ	1.0 ± 0.4	0.8 ± 0.7	0.5 ± 0.2*†	0.8 ± 0.4*
EZ	2.9 ± 0.8	3.4 ± 0.8	1.6 ± 0.7*†	1.8 ± 0.8*†

* $p < 0.05$ compared with Group 1.
† $p < 0.05$ compared with Group 2.

degradation rate of the material.[7,15,25,29] Although mild inflammatory reactions have been observed when using large amounts of polylactides, they are generally well tolerated.[17] In the use as a drug delivery system, the drug-release kinetics of the carrier is of fundamental importance to obtain a therapeutic concentration in tissue and to avoid toxic side concentrations. The drug release from the PDLLA used in this study takes place in two steps:[12] primarily by diffusion without mass loss, followed by erosion of the matrix.[41] Additionally, the degradation of copolymers depends on the relative amount of amorphous compared with crystalline polymer with increased degradation found in amorphous regions.[5,27,32] Therefore, semicristalline PDLLA homopolymer degrades at a slower rate than the amorphous PDLLA homopolymer. This may be the reason that some investigators have found a higher incidence of voids within the spinal fusion mass by using PDLLA carriers.[9]

The local application of bioactive molecules from biodegradable PLA or PGA systems were examined in several in vitro and in vivo studies. Gombotz, et al.,[11] reported that the controlled release of transforming growth factor–β1 derived from a biodegradable poly(D,L-lactide-co-glycolide acid) supported the bone ingrowth into the carrier system. Zegzula, et al.,[45] described a dose-dependent healing of bone defects treated with rhBMP-2 delivered on porous PDLLA implants in a rabbit model. Hermann, et al.,[14] demonstrated that the incorporation of

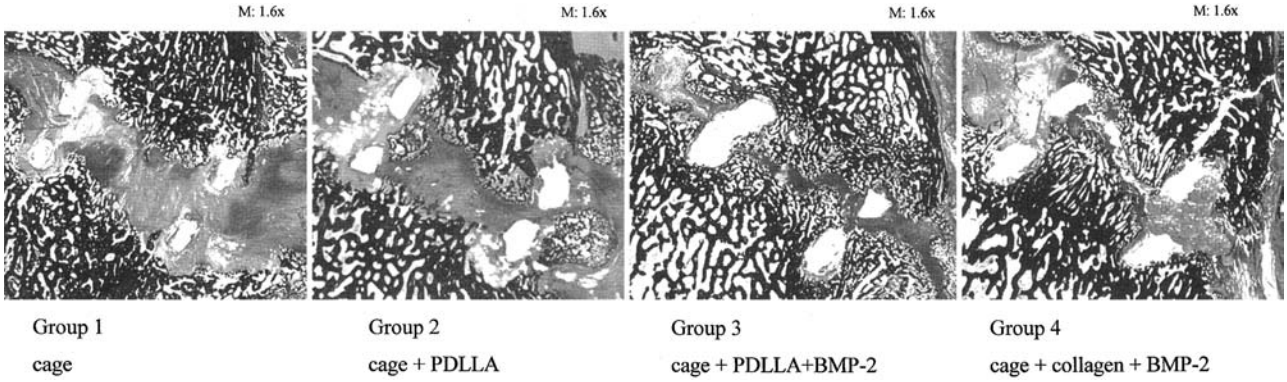


FIG. 3. Histomorphological analysis. After 12 weeks interbody fusion was evaluated histomorphically and histomorphometrically. Safranin-O/van Kossa staining, original magnification × 1.6.

TABLE 4

*Summary of histomorphometrical data obtained after 12 weeks**

Index	Group 1	Group 2	Group 3	Group 4
SDD (mm)	25.6 ± 1.2 (24.6–27.0)	25.9 ± 1.6 (24.3–27.8)	25.8 ± 1.4 (24.7–27.9)	25.8 ± 1.2 (24.5–27.4)
BV/TV (%)	38.3 ± 4.1 (26.4–52.1)	41.8 ± 3.2† (31.7–50.2)	44.2 ± 3.1†‡§ (34.2–52.8)	41.9 ± 2.1† (35.6–49.9)
CV/TV (%)	4.3 ± 2.4 (1.4–11.0)	4.4 ± 2.1 (1.9–9.2)	6.1 ± 2.8† (1.9–17.2)	5.9 ± 2.3† (2.1–14.5)
mCV/CV (%)	3.4 ± 1.8 (0.8–5.0)	3.6 ± 1.0 (1.9–5.8)	4.1 ± 1.2† (1.8–6.1)	4.2 ± 1.1† (1.6–5.7)

* BV/TV = bone volume/total volume ratio; CV/TV = cartilage volume/total volume ratio; mCV/CV = mineralized CV/CV ratio; SDD = sagittal diameter distance (baseline).
† $p < 0.05$ compared with Group 1.
‡ $p < 0.05$ compared with Group 2.
§ $p < 0.05$ compared with Group 4.

TABLE 5

*Summary of fluorochrome data obtained after 12 weeks**

	Group 1		Group 2		Group 3		Group 4	
Index	Adjacent	W/In	Adjacent	W/In	Adjacent	W/In	Adjacent	W/In
3 wks	1	1	0	0	2	2	2	2
6 wks	1	5	3	6	3	4	4	3
9 wks	1	6	5	6	8	8	8	8

* Depicted are the number of fusion sites (of the different groups at different time points) in which the fluorochrome marker was present adjacent to or within the cage or bone graft, respectively.

antithrombotic drugs in biodegradable PDLLA–coated coronary stents led to a significant reduction of local thrombotic reactions and restenosis of coronary vessels.

In our study a biodegradable PDLLA carrier system was used for application of growth factors. Authors of previous studies, especially those focused on PGA,[9] have shown that during degradation of polymers, breakdown products are formed that may alter the physiological environment.[28] Therefore, it may be possible that these products influence healing processes. Additionally, by demonstrating a loss of the coating from steel and titanium implants of less than 5%, the authors of an intramedullary implantation and extraction study in rat tibiae have demonstrated a high mechanical stability of the coating;[37] however, a dislodgment of the PDLLA coating during insertion of the cage might have been possible. To examine the influence of the PDLLA on spinal fusion, PDLLA-coated (Group 2) and noncoated cages (Group 1) were investigated. Preliminary analyses of blood, serum, body weight, and body temperature showed no significant difference between the two groups, suggesting the absence of PDLLA-related adverse systemic side effects. Additionally, no voids in the interbody fusion mass or inflammatory reaction associated with the PDLLA coating were found in histomorphological evaluations. No difference in the quantity of macrophages, especially the absence of macrophages or PDLLA remnants outside the intervertebral space indicating dislodgment of the PDLLA coating, were observed on the histological sections in any of the groups. Therefore, no local PDLLA carrier–associated side effects could be detected. In contrast to previous studies in rat tibia fractures,[37] however, we found no positive effect of the PDLLA carrier on bone matrix formation; yet, compared with the noncoated cages, all PDLLA-coated cages showed significantly higher values for BMD of the callus and bone volume/total volume ratio, as well as slightly higher values for radiographic osseous fusion parameters, functional radiographic evaluation of LA, BMC, BCV, ROM in flexion and extension, and new bone formation adjacent to the cage after 9 weeks. Therefore, in contrast to previous studies of biodegradable PGA or PLA carriers,[9] the PDLLA carrier may exert a positive effect on bone matrix formation; however, it could not be detected in this study, possibly because of the limited number of animals used.

Bone Morphogenetic Protein–2

In this study significant acceleration of interbody fusion was caused by BMP-2. Compared with the untreated cage group, significantly higher radiographic fusion rate, a higher biomechanical stability, an advanced interbody fusion on histomorphometric analysis, and an accelerated interbody fusion on fluorochrome sequence labeling were associated with PDLLA-coated BMP-2-treated cages. These results are in accordance with previous animal studies in which BMP-2 was used in experimental spinal fusion.[2,3,8,9,19,31,36,44]

In the majority of animal studies investigators have used BMP-2 in an intertransverse process spinal fusion model.[3,8,9,19,31,36] Compared with this model, the biological environment of anterior interbody fusion provides a greater access to cancellous bone and bone marrow elements with osteogenetic potency. This favorable biological environment may therefore reduce the quantity of growth factors needed to achieve solid fusion, making the use of growth factors more economically feasible. In several studies in which intertransverse fusion models were evaluated, high BMP-2 doses (up to 1500 μg) were necessary to achieve intertransverse fusion.[3,8,9,19,31,36] In our study a small dose of BMP-2 (approximately 150 μg) applied with a PDLLA-coated cage or a collagen sponge was able to accelerate anterior interbody bone matrix formation significantly.

Additionally, BMP-2 also induces de novo bone in ectopic soft-tissue sites even in the absence of bone marrow elements. In previous studies BMP-2-induced bone formation in the ligamentum flavum resulted in flattening of the spinal cord.[33] Hoshi, et al.,[16] were able to show that BMP-2 induced ossification of the spinal ligaments, which resulted in spinal cord compression. Other authors have suggested that BMP-2 may play an important role in the ossification of spinal ligaments, especially the posterior longitudinal ligament.[16,24] In our study anterior interbody discectomy and fusion was performed in which the posterior longitudinal ligament was preserved. In accordance with the results of Zdeblick, et al.,[44] and Hecht, et al.,[13] who also used an anterior interbody fusion model, we found no evidence of ossification of the posterior longitudinal ligament associated with the BMP-2. Additionally, no BMP-2-related systemic side effects on blood count, electrolytes, glucose levels, thyroid hormones, body weight, or body temperature were observed in this study.

In previous anterior interbody fusion models in which the surgeons used BMP-2, threaded cages (horizontal cylinders) were applied to stabilize the anterior spinal column.[2,13,44] In in vitro experiments involving sheep cervical spines our group[21] demonstrated profound biomechanical differences between threaded (horizontal cylinders) and cylindrical cages (vertical cylinder) such as the titanium mesh cage used in this study. Particularly in terms of bending, the vertical cylinder cages showed significantly higher stiffness and lower ROM values than the threaded cages. Nevertheless, we were not able to determine any relevant differences in interbody bone matrix formation when comparing the results of the BMP-2-treated cylindrical cages in this study with the BMP-2-treated threaded cages in the previous studies.[2,13,44] This suggests that the osteoinductive effect of BMP-2 applied in the intervertebral space is to a certain degree independent from the biomechanical properties of the added intervertebral implant.

Another purpose of this study was to compare a new PDLLA carrier system with a collagen sponge carrier. In comparison to BMP-2 application with a collagen sponge carrier, BMP-2 application with a PDLLA carrier resulted in a higher BCV and a higher bone volume/total volume ratio. No intergroup differences, however, were found for biomechanical parameters. These results are in accordance with those reported in previous studies. Zegzula, et al.,[45] have reported that the local application of rhBMP delivered via collagen sponges or bioabsorbable PLA/PGA beads with osteopromotive membranes into rat mandibular defects showed superior bone regeneration associated with PLA/PGA than with the collagen sponge carrier. In a study comparing bovine Type I collagen and polylactic acid as carriers for BMP-2 in a canine posterior intertransverse process fusion model, the investigators found a significantly higher axial rotation stiffness in the fusion produced with the polylactic acid carrier.[34] The fast release of drugs from collagen sponges[18] in combination with the quick inactivation of BMP-2 in vivo[39] resulted in a "short-time effect" of BMP-2. In contrast, high and continuously released BMP-2 concentrations can be obtained using a PDLLA carrier system. Therefore, in this study BMP-2 application with a PDLLA-coated cage resulted in a greater interbody fusion mass.

Conclusions

We found that PDLLA coating of cervical spine interbody fusion cages as a delivery system for growth factors was effective and safe. The PDLLA coating was not associated with any adverse effects on cervical spine interbody fusion. The slight but insignificant positive effect of the PDLLA carrier on interbody fusion might be a result of the degradation process of the biodegradable carrier.

Compared with the collagen sponge carrier, BMP-2 delivered via a PDLLA-coated interbody cage increased interbody bone matrix formation. In this new combination (implant + PDLLA plus growth factor) the cage was converted from an interbody cage to an interbody "fusion" cage because it served not only as a device for spinal fixation but also as a local drug delivery system.

Additional comparative in vivo studies of different growth factors and carriers might elucidate which growth factor or growth factor/carrier combination—also taking into account those which have not yet been tested—could create the basis for spinal fusion at optimum velocity and with minimal risks.

References

1. Aspenberg P, Turek T: BMP-2 for intramuscular bone induction: effect of squirrel monkeys is dependent on implantation site. **Acta Orthop Scand 67:**3–6, 1996
2. Boden SD, Martin GJ Jr, Horton WC, et al: Laparoscopic anterior spinal arthrodesis with rhBMP-2 in a titanium interbody threaded cage. **J Spinal Disord 11:**95–101, 1998
3. Boden SD, Martin GJ Jr, Morone MA, et al: Posterolateral lumbar intertransverse process spine arthrodesis with recombinant human bone morphogenetic protein 2/hydroxyapatite-tricalcium phosphate after laminectomy in the nonhuman primate. **Spine 24:**1179–1185, 1999
4. Boden SD, Zdeblick TA, Sandhu HS, et al: The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. **Spine 25:**376–381, 2000
5. Brady JM, Cutright DE, Miller RA, et al: Resorption rate, route, route of elimination, and ultrastructure of the implant site of polylactic acid in the abdominal wall of the rat. **J Biomed Mater Res 7:**155–166, 1973
6. Cloward RB: The anterior surgical approach to the cervical spine: the Cloward procedure: past, present, and future. The presidential lecture, Cervical Spine Research Society. **Spine 13:**823–827, 1988
7. Cutright DE, Hunsuck EE, Beasley JD: Fracture reduction using a biodegradable material, polylactic acid. **J Oral Surg 29:** 393–397, 1971
8. David SM, Gruber HE, Meyer RA Jr, et al: Lumbar spinal fusion using recombinant human bone morphogenetic protein in the canine. A comparison of three dosages and two carriers. **Spine 24:**1973–1979, 1999
9. Fischgrund JS, James SB, Chabot MC, et al: Augmentation of autograft using rhBMP-2 and different carrier media in the canine spinal fusion model. **J Spinal Disord 10:**467–472, 1997
10. Fujimoto A, Tanizawa T, Nishida S, et al: Local effects of transforming growth factor-beta1 on rat calvaria: changes depending on the dose and the injection site. **J Bone Miner Metab 17:** 11–17, 1999
11. Gombotz W, Pankey S, Bouchard L, et al: Controlled release of TGF-beta 1 from a biodegradable matrix for bone regeneration. **J Biomater Sci Polym Ed 5:**49–63, 1993
12. Gopferich A: Mechanisms of polymer degradation and erosion. **Biomaterials 17:**103–114, 1996
13. Hecht BP, Fischgrund JS, Herkowitz HN, et al: The use of recombinant human bone morphogenetic protein 2 (rhBMP-2) to promote spinal fusion in a nonhuman primate anterior interbody fusion model. **Spine 24:**629–636, 1999
14. Hermann R, Schmidmaier G, Markl B, et al: Antithrombogenic coating of stents using a biodegradable drug delivery technology. **Thromb Haemost 82:**51–57, 1999
15. Hollinger JO, Battistone GC: Biodegradable bone repair materials. Synthetic polymers and ceramics. **Clin Orthop 207:** 290–305, 1986
16. Hoshi K, Amizuka N, Sakou T, et al: Fibroblasts of spinal ligaments pathologically differentiate into chondrocytes induced by recombinant human bone morphogenetic protein-2: morphological examinations for ossification of spinal ligaments. **Bone 21:**155–162, 1997
17. Hutmacher D, Hurzeler MB, Schliephacke H: A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. **Int J Oral Maxillofac Implants 11:**667–678, 1996
18. Illi OE, Feldmann CP: Stimulation of fracture healing by local application of humoral factors integrated in biodegradable implants. **Eur J Pediatr Surg 8:**251–255, 1998

19. Itoh H, Ebara S, Kamimura M, et al: Experimental spinal fusion with use of recombinant human bone morphogenetic protein 2. **Spine 24:**1402–1405, 1999
20. Kandziora F, Kerschbaumer F, Klein C, et al: Biomechanical assessment of transoral plate fixation for atlantoaxial instability. **Spine 25:**1555–1561, 2000
21. Kandziora F, Pflugmacher R, Schäfer J, et al: Biomechanical comparison of cervical spine interbody fusion cages. **Spine 26:** 1850–1857, 2001
22. Kandziora F, Pflugmacher R, Scholz M, et al: Comparison between sheep and human cervical spines: an anatomic, radiographic, bone mineral density, and biomechanical study. **Spine 26:**1028–1037, 2001
23. Kleemann R: **Entwicklung eines Wirbelsäulenprüfstands zur Testung von Implantaten an der Halswirbelsäule.** Semesterarbeit. Fakultät für Maschinenbau. Technische Universität Berlin, 1999
24. Kon T, Yamazaki M, Tagawa M, et al: Bone morphogenetic protein-2 stimulates differentiation of cultured spinal ligament cells from patients with ossification of the posterior longitudinal ligament. **Calcif Tissue Int 60:**291–296, 1997
25. Kulkarni RK, Moore EG, Hegyeli AF, et al: Biodegradable poly(lactic acid) polymers. **J Biomed Mater Res 5:**169–181, 1971
26. Kulkarni RK, Pani KC, Neuman C, et al: Polylactic acid for surgical implants. **Arch Surg 93:**839–843, 1966
27. Kumta SM, Spinner R, Leung PC: Absorbable intramedullary implants for hand fractures. Animal experiments and clinical trial. **J Bone Joint Surg (Br) 93:**563–566, 1992
28. Laurencin C, Lane JM: Poly (lactide acid) and poly (glycolid acid): orthopedic surgery applications, in Brighton C, Frielaender G, Lane MJ (eds): **Bone Formation and Repair.** Rosemont, IL: American Academy of Orthopaedic Surgeons, 1994, pp 325–339
29. Majola A, Vainionpaa S, Vihtonen K, et al: Absorption, biocompatibility and fixation properties of polylactic acid in bone tissue: an experimental study in rats. **Clin Orthop 268:** 260–269, 1991
30. Martin GJ Jr, Boden SD, Marone MA, et al: Posterolateral intertransverse process spinal arthrodesis with rhBMP-2 in a nonhuman primate: important lessons learned regarding dose, carrier, and safety. **J Spinal Disord 12:**179–186, 1999
31. Meyer RA Jr, Gruber HE, Howard BA, et al: Safety of recombinant human bone morphogenetic protein-2 after spinal laminectomy in the dog. **Spine 24:**747–754, 1999
32. Miller RA, Brady JM, Cutright DE: Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. **J Biomed Mater Res 11:**711–719, 1977
33. Mimatsu K, Kishi S, Hashizume Y: Experimental chronic compression on the spinal cord of the rabbit by ectopic bone formation in the ligamantum flavum with bone morphogenetic protein. **Spinal Cord 35:**740–746, 1997
34. Ozuna R, Sandhu HS, Kanim LEA: Carriers of bone morphogenetic protein for posterolateral spinal fusion. **Proceedings of the North American Spine Society 10th Annual Meeting**, 1995, p 14
35. Saitoh H, Takata T, Nikai H, et al: Effect of polylactic acid on osteoinduction of demineralized bone: preliminary study of the usefulness of polylactic acid as a carrier of bone morphogenetic protein. **J Oral Rehabil 21:**431–438, 1994
36. Sandhu HS, Kanim LE, Kabo JM, et al: Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion. **Spine 21:**2115–2122, 1996
37. Schmidmaier G, Wildemann B, Bail H: Local application of growth factors (insulin-like growth factor-1 and transforming growth factor-beta1) from a biodegradable poly(D,L-lactide) coating of osteosynthetic implants accelerates fracture healing in rats. **Bone 28:**341–350, 2001
38. Schmidmaier G, Wildemann B, Stemberger A, et al: Biodegradable poly(D,L-lactide) coating of implants for continuous release of growth factors. **J Biomed Mat Res 58:**449–455, 2001
39. Sorensen TS, Sorensen AI, Merser S: Rapid release of gentamicin from collagen sponge. In vitro comparison with plastic beads. **Acta Orthop Scand 61:**353–356, 1990
40. Takaoka K, Koezuka M, Nakahara H: Telepeptide-depleted bovine skin collagen as a carrier for bone morphogenetic protein. **J Orthop Res 9:**902–907, 1991
41. Tamada JA, Langer R: Erosion kinetics of hydrolytically degradable polymers. **Proc Natl Acad Sci USA 90:**552–556, 1993
42. Terrell TG, Working PK, Chow CP, et al: Pathology of recombinant human transforming growth factor-beta 1 in rats and rabbits. **Int Rev Exp Pathol 34B:**43–67, 1993
43. Wilton P: Treatment with recombinant human insulin-like growth factor I of children with growth hormone receptor deficiency (Laron syndrome). Kabi Pharmacia Study Group on Insulin-like Growth Factor I Treatment in Growth Hormone Insensitivity Syndromes. **Acta Paediatr Suppl 383:**137–142, 1992
44. Zdeblick TS, Ghanayem AJ, Rapoff AJ, et al: Cervical interbody fusion cages. An animal model with and without bone morphogenetic protein. **Spine 23:**758–766, 1998
45. Zegzula HD, Buck DC, Brekke J, et al: Bone formation with use of rhBMP-2 (recombinant human bone morphogenetic protein-2). **J Bone Joint Surg (Am) 79:**1778–1790, 1997

Manuscript received June 11, 2001.
Accepted in final form February 25, 2002.
The work in this paper was supported by grant No. RS/KF-82042 from the Max Biedermann Institut der Steinbeis Stiftung.

Address reprint requests to: Frank Kandziora, M.D., Unfall- und Wiederherstellungschirurgie, Universitätsklinikum Charité der Humboldt Universität Berlin, Campus Virchow Klinikum, Augustenburgerplatz 1, 13353 Berlin, Germany. email: frank.kandziora@charite.de.

Diese Untersuchung konnte zeigen, dass der PDLLA-Carrier eine sichere und effektive lokale Applikation von Wachstumsfaktoren erlaubt. Zusätzlich konnte nachgewiesen werden, dass der PDLLA-Carrier im Vergleich zum Kollagen-Carrier eine Verbesserung der Ergebnisse der intervertebralen Spondylodese ermöglicht. Daher wurden alle folgenden Untersuchungen mit dem PDLLA-Carrier durchgeführt.

2.3.2 Dosisabhängige Wirksamkeit von IGF-I und TGF-β1

Die spinale Applikation von BMP-2 ist mit Nachteilen verbunden [81,106,133]. Alternativ machen die synergistischen Effekte von IGF-I und TGF-β1 auf die Knochenneubildung und –regeneration [83,137,181] deren kombinierte Applikation zur Stimulation der intervertebralen Spondylodese interessant. Da die kombinierte Applikation von IGF-I und TGF-β1 bisher noch nicht untersucht wurde, musste zunächst eine adäquate Dosis für diese Wachstumsfaktorenkombination definiert werden.

- Daher sollte in dieser Untersuchung zunächst geklärt werden, ob die kombinierte lokale Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage in der Lage ist, die intervertebrale Spondylodese zu stimulieren.
- Zusätzlich sollten in dieser Untersuchung 3 verschiedene Dosen von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage appliziert werden und hinsichtlich ihres Effekts auf die radiologische, biomechanische und histologische Einheilung intervertebraler Cages untersucht werden.

European Spine Journal 2003- published online 8.11.02

Dose-dependent effects of combined IGF-I and TGF-ß1 application in a sheep cervical spine fusion model

F. Kandziora , R. Pflugmacher, M. Scholz, J. Schäfer, G. Schollmeier,G. Schmidmaier, G. Duda, M. Raschke, and N. P. Haas

Introduction: Combined IGF-I and TGF-ß1 application by a Poly-(D,L-lactide)(PDLLA) coated interbody cage has proven to promote spine fusion. Purpose of this study was to determine, whether there is a dose-dependent effect of combined IGF- I and TGF-ß1 application on intervertebral bone matrix formation in a sheep cervical spine fusion model.

Method: 32 sheep underwent C3/4 discectomy and fusion. Stabilisation was performed using a titanium cage coated with a PDLLA carrier including no growth factors in group 1 (n = 8), 75 µg IGF-I plus 15 µg TGF-ß1 in group 2 (n = 8), 150 µg IGF-I plus 30 µg TGF-ß1 in group 3 (n = 8) and 300 µg IGF-I plus 60 µg TGF-ß1 in group 4 (n = 8). Blood samples, body weight and temperature were analysed. Radiographic scans were performed pre- and postoperatively and after 1, 2, 4, 8, 12 weeks, respectively. At the same time points, disc space height and intervertebral angle were measured. After 12 weeks animals were killed and fusion sites were evaluated using quantitative computed tomographic scans to assess bone mineral density, bone mineral content and bony callus volume. Biomechanical testing was performed and range of motion, neutral and elastic zone were determined. Histomorphological and histomorphometrical analysis were carried out and polychrome sequential labelling was used to determine the time frame of new bone formation.

Results: In comparison to the group without growth factors (group 1) the medium and high dose growth factor groups (group 3 and 4) demonstrated a significant higher bony callus volume on CT-scans, a higher biomechanical stability, an advanced interbody bone matrix formation in histomorphometric analysis, and an earlier bone matrix formation on fluorochrome sequence labelling. Additionally, the medium and high dose growth factor groups (group 3 and 4) demonstrated a significant higher bony callus volume, a higher biomechanical stability in rotation, and an advanced interbody bone matrix formation in comparison to the low dose growth factor group (group 2). No significant difference could be determined between the medium (group 3) and high dose growth factor group (group 4).

Conclusion: The local application of IGF-I and TGF-ß1 by a PDLLA coated cage significantly improved results of interbody bone matrix formation in a dose dependent manner. The best dose-response-relationship was achieved with the medium growth factor dose (150 µg IGF-I and 30 µg TGF-ß1). With an increasing dose of these growth factors no further stimulation of bone matrix formation was observed. Although these results are encouraging, safety issues of combined IGF-I and TGF-ß1 application for spinal fusion still have to be addressed.[Key words: cervical spine,sheep, animal model, interbody fusion, IGF-I, TGF-ß1, growth factor, surgery, dose]

From the Unfall- und Wiederherstellungschirurgie, Universitätsklinikum Charité der Humboldt Universität Berlin
Campus Virchow Klinikum

More than 30 years ago Urist [55] determined the osteoinductive capacity of demineralized bone matrix. Advances in protein isolation and molecular cloning technology subsequently yielded several soluble, low molecular weight growth factors, like transforming growth factors (TGF's), bone morphogenetic protein's (BMP's), platelet derived growth factors (PDGF's), insulin like growth factors (IGF's), fibroblast growth factors (FGF's) and epidermal growth factors (EGF's) [53]. Meanwhile, many of these growth factors are available as recombinant molecules in virtually unlimited quantities using genetically modified cell lines. However, only some of these growth factors have demonstrated a significant osteoinductive capacity and only two of this growth factors have been precisely evaluated in experimental spine fusion. Presently, only BMP-2 [4-6, 9, 12, 16, 22, 33, 34, 46, 47, 60, 61] and OP-1 (BMP-7) [15, 30] have proven to accelerate spinal fusion and to overcome the disadvantages of an autologous bone graft. However, provisos have been expressed due to side effects associated with the use of BMP´s in spinal fusion. BMP´s are able to induce de novo bone in ectopic soft tissue sites even in the absence of bone narrow elements [2, 61]. Some authors postulated that there is a necessity for a growth factor to induce ectopic bone to promote interbody fusion sufficiently [4, 33, 34]. However, this characteristic of BMP-2 or BMP-7 might also be harmful. In previous studies new bone formation induced by BMP-2 in the ligamentum flavum resulted in flattening of the spinal cord [35]. Additionally, Hoshi [19] was able to show that BMP-2 induced ossifications of the spinal ligaments lead even to spinal cord compression. Other authors suggested that the BMP´s might play an important role in the ossification of spinal ligaments, especially the posterior longitudinal ligament [19, 28]. Therefore, the optimum growth factor or growth factor combination to promote spinal fusion is still a matter of discussion.

In a former study we were able to show an increased intervertebral bone matrix formation comparing combined IGF-I and TGF-ß1 application with an autologous tricortical iliac crest bone graft in a sheep cervical spine interbody fusion model [26]. Additionally, we demonstrated analogous biomechanical and histological results comparing BMP-2 and combined IGF-I and TGF-ß1 application in this model [27]. Due to these results combined IGF-I and TGF-ß1 application seems to be very promising for bone induction in the field of spine surgery. However, to our knowledge, no studies have been performed to determine if a dose-dependent

effect of combined IGF-I and TGF-ß1 application on intervertebral fusion exists.
Therefore, purpose of this study was to determine, whether there is a dose-dependent effect of combined IGF- I and TGF-ß1 application on intervertebral bone matrix formation in a sheep cervical spine interbody fusion model. Further this study was designed, to define a dose which might be suitable for further experimental evaluation of this growth factor combination.

Material and Method

Study design

32 adult female merino sheep (2 years old) underwent C3/4 discectomy and fusion. The sheep were randomly assigned to the following groups:

Group 1: titanium cage coated with a biodegradable PDLLA carrier (n = 8);
Group 2: titanium cage coated with a biodegradable PDLLA carrier including IGF-I (2.5% w/w = 75 µg) and TGF-ß1 (0.5% w/w = 15µg) (n = 8); (low dose group);
Group 3: titanium cage coated with a biodegradable PDLLA carrier including IGF-I (5.0% w/w = 150 µg) and TGF-ß1 (1.0% w/w = 30 µg) (n = 8); (medium dose group);
Group 4: titanium cage coated with a biodegradable PDLLA carrier including IGF-I (10% w/w = 300 µg) and TGF-ß1 (2.0% w/w = 60 µg) (n = 8); (high dose group).

After 12 weeks all sheep were sacrificed and radiographic, biomechanical and histological evaluations were performed. All animal experimental work was approved by local authorities.

Coating of the cages

Poly-(D,L-lactide)(PDLLA, Boehringer Ingelheim, Germany) was chosen as drug carrier system. The properties of the PDLLA-coating and the coating technique were described previously [17, 48]. In groups 2-4 recombinant human insulin like growth factor I (IGF-I, R&D Systems) (2.5, 5 and 10% w/w) and recombinant human transforming growth factor-beta 1 (TGF-ß1, R&D Systems) (0.5, 1 and 2% w/w) were incorporated in the PDLLA-coating. The average PDLLA coating mass of the cages was 3.02 +/- 0.12 mg. There was no difference between the total coating mass of the different groups. Therefore, approximately 75, 150 and 300 µg IGF-I plus 15, 30 and 60µg TGF-ß1 were incorporated in the coating of each cage, respectively.

Surgical technique and postoperative care

The animals underwent the surgical procedure under general endotracheal anaesthesia. The anterior part of the neck was prepped in a sterile fashion and a left anterolateral approach to the cervical spine was carried out through a longitudinal skin incision. The longus colli muscle was incised in the midline, and the intervertebral disc C3/4 was exposed. After distraction of the motion segment with a Caspar-distractor, anterior discectomy C3/C4 was performed. The endplates were uniformly shaved with a 2 mm high speed diamond drill down to bleeding bone. For interbody stabilization the coated meshed titanium cages (Motech GmbH, Schwenningen, Germany, height 8 mm, diameter 14 mm) including the growth factors were inserted uniformly into the intervertebral space. Finally, the wound was irrigated with saline and the longus colli muscle, the subcutaneous tissue and the skin were reapproximated with sutures and a soft bandage was applied to the neck.
After surgery, the animals were maintained under observation until fully recovered from general anaesthesia. They received two doses of 0.5 g metamizol-natrium (Novaminsulfon ®, Lichtenstein) per day for 5 days intramuscularly. Clinical examination was performed daily for the first 10 days, then weekly. The sheep were allowed ad libitum activity for the remainder of the experiment. Fluorochrome sequential labels were administered at 3, 6, and 9 weeks postoperatively consisting of oxytetracyclin (25 mg/kg IV) at 3 weeks, calcein green (15 mg/kg IV) at 6 weeks, and xylenol orange (90 mg/kg IV) at 9 weeks. 12 weeks after surgery, the animals were killed after induction of anaesthesia by an intravenous injection of potassium chloride. The complete cervical spine including parts of the occiput and Th 1 was then excised and cleaned from the surrounding tissue.

Blood and serum analysis

Blood and serum samples were taken from the saphenous vein of the hind leg of the sheep pre- and postoperatively and after 1, 2, 4, 8, 12 weeks, respectively. The blood samples were analysed for routine laboratory parameters (blood count, electrolytes, alkaline phosphatase, thyroid values and glucose).

Body weight and body temperature analysis

Preoperatively and after 1, 2, 4, 8, 12 weeks rectal body temperature and body weight were determined.

Radiographic analysis

Radiographic evaluations have been described in detail earlier [24]. Lateral and p.a. digital radiographic scans (X-ray unit: Mobilett Plus, Siemens AG, Germany; X-ray films: Fuji CR 24x30, Fuji, Germany) were performed pre- and postoperatively and after 1, 2, 4, 8, 12 weeks, respectively. At the same time periods anterior, middle and posterior intervertebral disc space heights (DSH) and intervertebral angle (IVA) of the motion segment C3/C4 were measured on lateral radiographic scans. Average intervertebral DSH was calculated from anterior, middle and posterior DSH measurements (anterior, middle and posterior DSH / 3). All radiographic measurements were evaluated by three independent observers.

Quantitative computed tomographic analysis

Quantitative computed tomographic scans (QCT) were performed using a Siemens Somatom plus 4 scanner (Siemens Inc., Erlangen, Germany). Axial cuts with 1 mm slice thickness were made parallel to the intervertebral disc space. Bone mineral density (BMD) and bone volume measurements of the callus have been described in detail earlier [24]. BMD measurements were calibrated with a 6-point bone mineral density phantom and were performed using specific software of the scanner (Sienet Magic View VA 30A, Siemens, Inc., Erlangen, Germany). Bony callus volume (BCV) was measured using an image analysing system (Zeiss KS 400, Zeiss GmbH, Germany). Bone mineral content (BMC) was calculated from BMD and BCV measurements (BMC = BCV x BMD). All CT-

measurements were evaluated by three independent observers.

Biomechanical analysis

After euthanasia biomechanical testing was performed by a non-destructive flexibility method using a nonconstrained testing apparatus described in detail earlier [23-25]. Pure bending moments of 6 Nm load were applied to the motion segments C3/4 using a system of cables and pulleys to induce flexion, extension, left and right lateral bending and left and right axial rotation. Tension was applied to the cables with an uniaxial testing machine (1456, Zwick GmbH, Ulm, Germany). Three-dimensional displacement of each motion segment was measured using an optical measurement system (Qualysis Inc., Sävebalden, Sweden). Triangular markers with 3 diodes (Qualysis Inc., Sävebalden, Sweden) were attached to the bodies of C3 and C4. Marker positions were detected with two cameras and recorded with a computerized motion analysis system (PC-Reflex, Qualysis Inc., Sävebalden, Sweden). Angular displacement of the upper vertebra (C3) in relation to the lower vertebra (C4) was calculated from marker positions using custom-made computer software. The measurement error associated with this method was +/- 0.1 degrees [25]. Range of motion (ROM), neutral (NZ) and elastic (EZ) zones were determined.

Histomorpological, histomorphometrical and fluorochrome analysis

All C3/C4 motion segments were harvested at 12 weeks for bone histology. The motion segments had been fixed for 7 days in 10 % normal buffered formaldehyde followed by dehydration in ascending concentrations of ethanol and embedded undecalcified in methylmetacrylate (Technovit 9100, Heraeus Kulzer GmbH, Germany).

For histomorphological and histomorphometrical analysis longitudinal sections in the sagittal plane were cut at 6 µm with a Leica SM 2500S microtome and a 40 ° stainless steel knife. Afterwards the residual parts of the cages were removed and the following stains were used: a) Safranin-O/Lightgreen, b) Safranin-O/v. Kossa c) Astrablue and d) Masson-Goldner.

Masson-Goldner stainings were used for histomorphological analysis. Histomorphometrical parameters were measured on Safranin-O/Lightgreen, Safranin-O/v. Kossa and Astrablue stainings using a Leica DM-RB microscope and an image analysing system (Zeiss KS 400, Zeiss GmbH, Germany). Parameters were measured at a magnification of 1.6x.

The sagittal diameter distance (S) of C3 and the average preoperative DSH were determined to define the size of the region of interest (ROI) for histomorphometrical evaluation [26, 27]. The complete intervertebral fusion area was included in this ROI. The following structural indices were calculated in the ROI: bone volume / total volume (BV/TV), cartilage volume / total volume (CV/TV), mineralised cartilage volume / cartilage volume (mCV/CV).

For fluorochrome analysis longitudinal sections in the parasagittal plane were cut at 400 µm with a precise macro grinding machine (Fa. Exact, Norderstedt, Germany). These slices were then ground to a thickness of 80 µm using a precise micro grinding machine (Fa. Exact, Norderstedt, Germany). Fluorochrome markers were analysed under appropriate lightning conditions using a Leica DM-RB microscope and an image analysing system (Zeiss KS 400, Zeiss GmbH, Germany). Parameters were measured at a magnification of 1.6x.

Fluorochrome analysis of intervertebral fusion areas was described in detail earlier [60]. The first appearance of the marker served to time formation of new bone matrix. The presence or absence of each marker around or within the cage was used to determine the relative time frame of new bone formation.

Statistical analysis

Comparison of data was performed using one way ANOVA for independent samples followed by TUKEY post-hoc analysis for multiple comparison procedures with Bonferroni correction for multiple measurements. Intraobserver variability for radiographic, functional radiographic evaluation and CT-measurements was determined using kappa statistics. Statistical significant differences were defined at a 95% confidence level. The values are given as mean +/- standard deviation. SPSS (release 10.0, SPSS Inc. Chicago, Illinois) software supported statistical evaluation.

Results

Blood and serum analysis results

Full blood count did not show any significant differences between the groups and throughout the experiment. Levels of electrolytes (Na, K, Cl, Ca) did not show significant changes during the experimental period. Furthermore no differences of thyroid hormones, alkaline phosphatase and glucose levels were found throughout the observation period and between all groups.

Body weight and body temperature

No significant differences were found between all groups in mean body temperature and body weight throughout the experimental period. Postoperatively, a slight and constant increase in body weight for all sheep of all groups was determined.

Radiographic results

Intraobserver agreement for radiographic measurements was good, showing kappa values ranging between 0.78 and 0.94. Preoperative baseline values of all radiographic parameters did not show any differences between the groups. No significant differences were found for average disc space height and intervertebral angle between all groups throughout the observation period. After a slight postoperative increase of disc space height and intervertebral angle due to intervertebral cage stabilization, these parameters decreased to preoperative values within 2 weeks. After these first 2 weeks no significant further loss of disc space height and intervertebral angle occurred.

Quantitative computed tomographic results

Intraobserver agreement for CT measurements was excellent, showing kappa values ranging between 0.88 and 0.98. After 12 weeks bony callus volume (BCV) was significantly lower in the cage group without growth factors (group 1) than in any other group ($p<0.05$) (table 1). Additionally, the medium and high dose growth factor groups (group 3 and 4) showed a significantly ($p<0.05$) higher BCV than the low dose growth factor group (group 2). There were no significant differences in bone mineral content (BMC) between the cage group without growth factors (group 1) and the low dose growth factor group (group 2). However, the medium and high dose growth factor groups (group 3 and 4) demonstrated a significantly ($p<0.05$) higher BMC than both other

groups (group 1 and 2). There was no significant difference for bone mineral density of the callus (BMD) between all groups.

Biomechanical results

Biomechanical results for range of motion (ROM), neutral zone (NZ) and elastic zone (EZ) are depicted in table 2. Lowest ROM, NZ and EZ values were constantly found for the high dose growth factor group (group 4). ROM in all directions, except flexion, and NZ and EZ in rotation and lateral bending were significantly ($p<0.05$) lower in the medium and high dose growth factor groups (group 3 and 4) than in the cage group without growth factors (group 1). No significant difference for ROM, NZ and EZ in any direction was found between the cage without growth factor group (group 1) and the cage plus low dose growth factors (group 2). Additionally, no significant difference was found between the medium (group 3) and high (group 4) dose growth factor group. In comparison to the low dose growth factor group (group 2), the medium and high dose growth factor groups (group 3 and 4) showed a significantly ($p<0.05$) lower ROM in rotation and lateral bending and a significantly ($p<0.05$) lower NZ and EZ in rotation.

Histomorphological results

Histomorphological analysis supported the findings of computed tomographic and biomechanical examinations (figure 1a-d). In the cage plus PDLLA group (group 1) mainly fibroblasts and occasionally cartilage cells were observed between the endplates. Cages were surrounded by a distinct small line of fibroblasts interrupted by some small bony islands. Group 2 stabilised with cages including a low dose of IGF-I and TGF-ß1, showed some bony islands between the endplates with cartilage and fibrous tissue components. The tissue surrounding the cages appeared similar to group 1. Group 3 and 4 stabilised with cages including medium and high doses of IGF-I and TGF-ß1, showed extensive callus formation and large bony islands or complete bony bridging between the endplates with little cartilage and fibrous tissue components. Most of the callus was seen inside the cage. These findings were accompanied by capillary ingrowth and small resorptive lacunae without major differences between both groups. Cages were surrounded by bone, however, in some areas a distinct small line of fibroblasts was obvious. Beside that, no ossifications of the spinal ligaments were investigated in any group.

Histomorphometrical results

The results of histomorphometrical analysis are presented in table 3. Histomorphometrical analysis showed no significant differences of sagittal diameter index (baseline) between all groups. Compared to the cage without growth factor group (group 1) and the low dose growth factor group histomorphometrical parameters revealed a significantly ($p<0.05$) higher bone volume/total volume ratio in the medium and high dose growth factor groups (group 3 and 4). No differences in the bone volume/total volume ratio were found between the medium (group 3) and high dose (group 4) growth factor group. There were no differences in the other histomorphometrical parameters for all groups.

Flurochrome analysis results

The results of fluorochrome analysis are depicted in table 4. The IGF-I/TGF-ß1 coated cages (group 2, 3 and 4) exhibited earlier new bone formation both within and around the cages compared to the group without growth factors (group 1). There were no differences in fluorochrome marker appearance between group all groups after 6 and 9 weeks.

Discussion

Recently, in vitro and in vivo studies have demonstrated an osteoinductive effect of IGF- I and TGF-ß1 application by direct and indirect mechanisms [31, 32, 37, 51, 52, 53]. IGF-I stimulates angiogenesis, the replication of osteoblasts and the synthesis of bone matrix [18, 21, 52]. TGF-ß1 regulates differentiation and proliferation of different cell types that are directly involved in bone remodeling and bone matrix formation, like mesenchymal cells, chondrocytes, osteoblasts, and osteoclasts [29, 43, 45]. In vivo studies have shown that decreased levels of IGF-I or TGF-ß1 are associated with bone loss and osteoporosis [1, 56, 58] whereas the local application of IGF-I or TGF-ß1 can positively influence fracture healing [20, 39, 50]. In contrast to the BMP´s, IGF-I and TGF-ß1 are said to be unable to induce de novo bone in the absence of bone narrow elements [49, 50]. Therefore, both growth factors might be able to overcome the disadvantages, especially the ossification of spinal ligaments, associated with the use of BMP´s in experimental spinal fusion.

Furthermore, in vitro and in vivo studies have demonstrated an significant osteoinductive effect combining IGF-I with growth factor's of the TGF-superfamily [21, 26, 27, 49, 59]. Illi [21] for example, combined the application of IGF-I with BMP-2 in an osteotomy model of the metacarpal bone in juvenile calves. Yeh [59] demonstrated, that combined OP-1 (BMP-7) and IGF-I application has a synergistic stimulatory effect on fetal rat calvaria cells, by OP-1 induced regulation of IGF-binding proteins (IGFBP´s). Schmidmaier [49] demonstrated, that the combined application of IGF-I and TGF-ß1 had a significantly higher stimulating effect on bone matrix formation in rat tibia fractures than single application of IGF-I or TGF-ß1. He concluded, that both growth factors had synergistic effects on fracture healing. Similar conclusions were drawn by other authors [36, 42].

These synergistic effects of IGF-I and TGF-ß1 have been explained by interactions between the IGF- and TGF-systems [8, 11, 54, 57]. Tremollieres [54] demonstrated, that TGF-ß1 is a potent modulator of IGF-I secretion. Kveiborg [29] showed, that TGF-ß1 exerts a significant stimulatory effect on the IGF-system, especially IGFBP-3, and concluded that this might represent a mechanism mediating TGF-ß1 effects on the biological functions of cells. Additionally, a feedback mechanism between IGFBP-6 and TGF-ß1 regulating the bone microenvironment was assumed [14]. Finally, O´Keefe [40] demonstrated, that IGF-I was able to augment the effects of TGF-ß1 on cartilage cells.

Due to these synergistic effects, the combined application of IGF-I and TGF-ß1 seems to be very promising for induction of bone matrix formation in the field of spine surgery. Additionally, in vivo studies have already demonstrated a significant osteoinductive effect of combined IGF-I and TGF-ß1 application in a sheep cervical spine interbody fusion model [26, 27]. Kandziora [26] was able to show an increased biomechanical stiffness and an accelerated intervertebral bone matrix formation comparing combined IGF- I and TGF-ß1 application with a autologous tricortical iliac crest bone graft. Additionally, he demonstrated analogous osteoinductive effects comparing similar doses of BMP-2 and combined IGF-I and TGF-ß1 application [27]. The

results of these previous studies are in concordance with the results presented in this study. In comparison to the cage without growth factors (group 1) the medium and high dose growth factor groups (group 3 and 4) demonstrated a significant higher bony callus volume on CT-scans, a higher biomechanical stability, an advanced interbody bone matrix formation in histomorphometric analysis, and an earlier interbody bone matrix formation on fluorochrome sequence labelling. Although the medium and high dose growth factor groups (group 3 and 4) showed an earlier and accelerated interbody bone matrix formation in comparison to the group without growth factors (group 1), the loss of disc space height and lordosis (intervertebral angle) was similar in all these groups. Therefore, especially in the first two weeks loss of disc space height seems to be primarily related to the interbody implant and widely independent from the amount of new formed bone.

For isolated application of IGF-I or TGF-ß1, a positive correlation between increasing dose and stimulatory effects on bone matrix formation has already been described [3, 13]. IGF-I and TGF-ß1 have dose-dependent effects on the activity of enzymes associated with bone matrix mineralisation [7, 44]. Panagakos [41] showed, that TGF stimulated a dose-dependent increase in chemotaxis of osteoblasts. Fujimoto [13] and Noda [38] demonstrated, that in vivo effects of TGF-ß1 on bone matrix formation in rat varied depending on concentration. However, both authors also determined nearly similar osteoinductive effects of TGF-ß1 in a wide range of concentrations [13, 38]. Additionally, Ebeling [10] demonstrated analogous effects of 4 different doses of IGF-I on bone turnover in normal women. Although dose-dependent effects for isolated IGF-I and TGF-ß1 application are sufficiently documented, to our knowledge, no study has been performed to determine, if a dose-dependent effect of combined IGF-I and TGF-ß1 application on bone matrix formation exists.

Currently, the "ideal" concentrations to induce spinal fusion for any growth factors are unknown. This is due to the fact, that each growth factor or growth factor combination has to be adapted to the local biological environment (anterior or posterior spinal fusion) and to the used carrier system. Some anterior spinal fusion models have shown good results with BMP-2 doses ranging between 100 and 250 µg [4, 16, 60]. Other studies [27] demonstrated, that equal doses of BMP-2 and IGF-I/TGF-ß1 showed nearly similar osteoinductive capacities. Additionally, the 5:1 ratio of IGF-I and TGF-ß1 applied by a PDLLA-coated implant has proven to be most effective [26, 27, 49]. Therefore, 3 different doses of a 5:1 ratio of IGF-I and TGF-ß1 were evaluated in this anterior interbody fusion model.

In comparison to the low dose growth factor group (group 2) the medium and high dose growth factor group group 3 and 4) demonstrated a significant higher bony callus volume on CT-scans, a higher biomechanical stability in rotation, and an advanced interbody bone matrix formation (bone volume / total volume ratio) in histomorphometric analysis. Although there was no difference between the low dose growth factor group (group 2) and the medium and high dose growth factor group (group 3 and 4) in the appearance of fluorochrome markers using fluorochrome sequence labelling, the amount of new formed bone was significantly higher with higher growth factor doses. However, no significant difference could be determined between the medium (group 3) and high dose growth factor group (group 4). Additionally, hardly any difference could be determined between the low dose growth factor group (group 2) and the group without growth factors (group 1). Therefore, the best dose-response-relationship in this study was achieved with the medium growth factor dose (150 µg IGF-I plus 30 µg TGF-ß1). As a further result of this study, the effective dose of IGF-I and TGF-ß1 in this interbody fusion model could be defined between 75 and 150 µg IGF-I and 15 and 30 µg TGF-ß1, respectively. With increasing dose of these growth factors no further stimulation of bone matrix formation could be achieved. Due to the fact that no difference in blood and serum analysis, body weight and temperature and no acute illness of the sheep could be determined in this study, the lethal doses for local combined IGF-I and TGF-ß1 application must be significantly higher than 300 µg IGF-I plus 60 µg TGF-ß1.

Currently, the reasons for these dose dependent effects of combined IGF-I an TGF-ß1 application on bone matrix formation in this sheep cervical spine fusion model are unknown. However, due to the interaction of the IGF-I and TGF-systems some inhibitory feedback mechanism activated with increasing doses of this growth factors might be possible.

Conclusion

The local application of IGF-I and TGF-ß1 by a PDLLA coated cage significantly improved results of interbody bone matrix formation in this sheep cervical spine fusion model. The best dose-response-relationship was achieved with the medium growth factor dose (150 µg IGF-I plus 30 µg TGF-ß1). With a increasing dose of these growth factors no further stimulation of bone matrix formation was observed. The effective dose of combined IGF-I and TGF-ß1 application could be defined between 75 and 150 µg IGF-I, and 15 and 30 µg TGF-ß1, respectively. Although these results are encouraging, further studies are essential to determine safety issues of combined IGF-I and TGF-ß1 application for spinal fusion.

Literature

1. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R. Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and oestrogen deficiency. J Bone Miner Res 2000; 15:683-69
2. Aspenberg P, Turek T. BMP-2 for intramuscular bone induction. Effect of squrrel monkeys is dependent on implantation site. Acta Orthop Scand 1996; 67:3-6
3. Beck L, Amento E, Xu Y, Deguzman L, Lee W, Nguyen T, Gillet N. TGF-beta 1 induces bone closure of skull defects – Temporal dynamics of bone formation in defects exposed to rhTGF-beta 1. J Bone Miner Res 1993; 8:753-761
4. Boden SD, Martin GJ Jr, Horton WC, Truss TL, Sandhu HS. Laproscopic anterior spinal arthrodesis with rh BMP-2 in a titanium interbody threaded cage. J Spinal Disord 1998; 11:95-101
5. Boden SD, Martin GJ Jr, Morone MA, Ugbo JL, Moskovitz PA. Posterolateral lumbar intertransverse process spine arthrodesis with recombinant human bone morphogenetic protein 2/hydroxyapatite-tricalcium phosphate after laminectomy in the nonhuman primate. Spine 1999; 24:1179-1185
6. Boden SD, Zdeblick TA, Sandhu HS, Heim SE. The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine 2000; 25:376-381
7. Bonewald LF, Schwartz Z, Swain LD, Ramirez V, Poser J, Boyan I. Stimulation of plasma membrane and matrix vesicle enzyme activity by transforming growth factor-beta in osteosarcoma cultures. J Cell Physiol 1990; 145: 200-206
8. Canalis E, Pash J, Gabbitas B, Rydziel S, Varghese S. Growth factors regulate the synthesis of insulin-like growth factor-I in bone cell cultures. Endocrinology 1993; 133: 33-38
9. David SM, Gruber HE, Mayer RA Jr, Murakami T, Tabor OB, Howard BA, Wozney JM, Hanley EN Jr. Lumbar spinal fusion using recombinant human bone morphogenetic protein in the canine. A comparison of three dosages and two carriers. Spine 1999; 24:1973-1979
10. Ebeling PR, Jones JD, O´Fallon WM, Janes CH, Riggs BL. Short-term effects of recombinant human insulin-like growth factor I on bone turnover in normal women. J Clin Endocrinol Metab 1993; 77: 1384-1387
11. Elford PR, Lamberts SW. Contrasting modulation by transforming growth factor-beta 1 of insulin like growth factor-I production in osteoblasts and chondrocytes. Endocrinology 1990; 127: 1635-1639
12. Fischgrund JS, James SB, Chabot MC, Hankin R, Herkowitz HN, Wozney JM, Shirkhoda A. Augmentation of autograft using rhBMP-2 and different carrier media in the canine spinal fusion model. J Spinal Disord 1997; 10:467-472
13. Fujimoto A, Tanizawa T, Nishida S, Yamamoto N, Soshi S, Endo N, Takahashi HE. Local effects of transforming growth factor-beta 1 on rat calvaria: changes depending on the dose and the injection site. J Bone Miner Metab 1999; 17:11-17
14. Gabbitas B, Canalis E. Growth factor regulation of insulin-like growth factor binding protein-6 expression in osteoblasts. J Cell Biochem 1997; 66; 77-86
15. Grauer JN, Patel TC, Erulkar JS, Troiano NW, Panjabi MM, Friedlaender GE. 2000 Young Investigator Research Award winner. Evaluation of OP-1 as a graft substitute for intertransverse process lumbar fusion. Spine 2001; 26:127-133
16. Hecht BP, Fischgrund JS, Herkowitz HN, Penman L, Toth JM, Shirkhoda A. The use of recombinant human bone morphogenetic protein 2 (rhBMP-2) to promote spinal fusion in a nonhuman primate anterior interbody fusion model. Spine 1999; 24:629-636
17. Hermann R, Schmidmaier G, Markl B, Resch A, Hahnel I, Stemberger A, Alt E. Antithrombogenic coating of stents using a biodegradable drug delivery technology. Thromb Haemost 1999; 82:51-57
18. Hock J, Centrella M, Canalis E. Insulin like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology 1998; 122:254-260
19. Hoshi K, Amizuka N, Sakou T, Kurokawa T, Ozawa H. Fibroblasts of spinal ligaments pathologically differentiate into chondrocytes induced by recombinant human bone morphogenetic protein-2:morphological examinations for ossification of spinal ligaments. Bone 1997; 21:155-162
20. Isgaard J, Nilson A, Lindahl A, Jansson J, Isaksson O. Effects of local administration of GH and IGF-I on longitudinal bone growth in rats. Am J Physiol 1986; 250:367-372
21. Illi OE, Feldmann CP. Stimulation of fracture healing by local application of humeral factors integrated in biodegradable implants. Eur J Pediatr Surg 1998; 8: 251-255
22. Itoh H, Ebara S, Kamimura M, Tateiwa Y, Kinoshita T, Yuzawa Y, Takaoka K. Experimental spinal fusion with use of recombinant human bone morphogenetic protein 2. Spine 1999 ; 24:1402-1405
23. Kandziora F, Kerschbaumer F, Starker M, Mittlmeier T. Biomechanical assessment of transoral plate fixation for atlantoaxial instability. Spine 2000; 25:1555-1561
24. Kandziora F, Pflugmacher R, Scholz M, Schnake K, Schröder R, Mittlmeier T. Comparison between sheep and human cervical spines: an anatomic, radiographic, bone mineral density, and biomechanical study. Spine 2001; 26:1028-37
25. Kandziora F, Pflugmacher R, Schäfer J, Duda G, Haas NP, Mittlmeier T. Biomechanical comparison of cervical spine interbody fusion cages. Spine 2001; 26: 1850-1857
26. Kandziora F, Schmidmaier G, Schollmeier G, Bail H, Pflugmacher R, Görke T, Wagner M, Raschke M, Mittlmeier T, Haas NP. IGF-I and TGF-ß1 application by a poly-(D,L-lactide) coated cage promotes intervertebral bone matrix formation in the sheep cervical spine. Spine 2002; 27 – in press
27. Kandziora F, Pflugmacher R, Scholz M, Knispel C, Hiller T, Schiollmeier G, Bail H, Schmidmaier G, Duda G, Raschke M, Haas NP. Comparison of BMP-2 and combined IGF-I/TGF-ß1 application in a sheep cervical spine fusion model. Eur Spine J 2002; - in press
28. Kon T, Yamazaki M, Tagawa M, Goto S, Terakado A, Moriya H, Fujimura S. Bone morphogenetic protei-2 stimulates differentiation of cultured spinal ligament cells from patients with ossification of the posterior longitudinal ligament. Calcif Tissue Int 1997; 60:291-296
29. Kveiborg M, Flyvbjerg A, Eriksen EF, Kaasem M. Transforming growth factor beta 1 stimulates production of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in human bone marrow stromal osteoblast progenitors. J Endocrinol 2001; 169: 549-561
30. Laursen M, Hoy K, Hansen ES, Gelineck J, Christensen FB, Bunger CE. Recombinant bone morphogenetic protein-7 as an intracorporal bone growth stimulator in unstable thoracolumbar burst fractures in humans: preliminary results. Eur Spine J 1999 ; 8:485-490
31. Lind M. Growth factor stimulation on bone healing. Effects on osteoblasts, osteotomies, and implants fixations. Acta Orthop Scand Suppl 1998; 283:2-37
32. Lind M, Schuhmacker B, Soballe K, Keller J, Melson F, Bunger C. Transforming growth factor-beta enhances fracture healing in rabbit tibiae. Act Orthop Scand 1993; 64:553-556
33. Martin GJ Jr, Boden SD, Marone MA, Marone MA, Moskovitz PA. Posterolateral intertransverse process spinal arthrodesis with rhBMP-2 in a nonhuman primate: important lessons learned regarding dose, carrier, and safety. J Spinal Disord 1999; 12:179-186
34. Meyer RA Jr, Gruber HE, Howard BA, Tabor OB Jr, Murakami T, Kwiatkowski TC, Wozney JM, Hanley EN Jr. Safety of recombinant human bone morphogenetic protein-2 after spinal laminectomy in the dog. Spine 1999; 24:747-754
35. Mimatsu K, Kishi S, Hashizume Y. Experimental chronic compression on the spinal cord of the rabbit by ectopic

bone formation in the ligamantum flavum with bone morphogenetic protein. Spinal Cord 1997; 35:740-746
36. Ma ZJ, Misawa H, Yamaguchi m. Stimulatory effect of zinc on insulin-like growth and transforming growth factor beta 1 production with bone growth of new-born rats. Int J Mol Med 2001; 8: 623-628
37. Mohan S, Baylink D. Bone growth factors. Clin Orthop Rel Res 1991; 263:30-48
38. Noda M, Camilliere JJ. In vivo stimulation of bone formation by transforming growth factor-beta. Endocrinology 1989; 124: 2991-2994
39. Nielson H, Isgaard J, Lindahl A, Peterson L, Isaksson O. Effects of unilateral arterial infusion of GH and IGF-I on tibial longitudinal bone growth in hypophysectomized rats. Calcif tissue Int 1987; 40:91-96
40. O´Keefe RJ, Crabb ID, Puzas JE, Rosier RN. Effects of transforming growth factor-beta 1 and fibroblast growth factor on DNA synthesis in growth plate chondrocytes are enhanced by insulin-like growth factor-I. J Orthop Res 1994; 12: 299-310
41. Panagakos FS. Transforming growth factor alpha stimulates chemotaxis of osteoblasts and osteoblast-like cells in vitro. Biochem Mol Biol Int 1994; 33: 643-650
42. Pfeilschifter J, Laukhuf F, Muller Beckmann B, Blim WF, Pfister H, Ziegler R. Parathyroid hormone increases the concentration of insulin-like growth factor-I and transforming growth factor beta 1 in rat bone. J Clin Invest 1995; 96: 767-774
43. Pfeilschifter J, Oechsner M, Naumann A, Gronwald R, Minne H, Ziegler R. Stimulation of bone matrix apposition in vitro by local growth factors: a comparison between insulin- like growth factor I, platelet derived growth factor and transforming growth factor beta. Endocrinology 1990; 127:69-75
44. Richman C, Baylink DJ, Lang K, Dony C, Mohan S. Recombinant human insulin-like growth factor-binding protein 5 stimulates bone formation parameters in vitro and in vivo. Endocrinology 1999; 140: 4699-4705
45. Roberts A, Sporn M, Bolander M. Transforming growth factor-beta and the initiation of chondrogenesis in the rat femur. J Cell Biol 1990; 110:2195-2207
46. Sandhu HS, Kanim LE, Kabo JM, Toth JM, Zeegen EN, Liu D, Delamater RB, Dawson EG. Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion. Spine 1996; 21:2115-22
47. Sandhu HS, Kanim LE, Toth JM, Kabo Jm, Liu D, Delamarter RB, Dawson EG. Experimental spinal fusion with recombinant human bone morphogenetic protein-2 without decortication of osseous elements. Spine 1997; 22:1171-1180
48. Schmidmaier G, Wildemann B, Stemberger A, Haas NP, Raschke M. Biodegradable poly-(D,L-lactide) coating of implants for contionous release of growth factors. J Biomed Mat Res 2001; 58:449-455
49. Schmidmaier G, Wildemann B, Stemberger A, Haas NP, Raschke M. Local application of growth factors (insulin-like growth factor-1 and transforming growth factor-beta1) from a biodegradable poly(D,L-lactide) coating of osteosynthetic implants accelerates fracture healing in rats. Bone 2001; 28: 341-350
50. Steinbrech DS, Mehrara BJ, Rowe NM, Dudziak ME, Luchs JS, Saadeh PB, Gittes GK, Longaker MT. Gene expression of TGF-beta, TGF-beta receptor, and extracellular matrix proteins during membranous bone healing in rats. Plast Reconstr Surg 2000; 105:2028-38
51. Terrell TG, Working PK, Chow CP, Green JD. Pathology of recombinant human transforming growth factor-beta 1. in rats and rabbits. Int Rev Exp Pathol 1993; 34B:43-67
52. Thaller S, Hoyt J, Tesluck H, Holmes R. The effect of insulin growth factor – I on calvarial sutures in Sprague-Dawley rat. J Craniofac Surg 1993; 4:35-39
53. Trippel S, Coutts R, Einhorn T, Mundy R, Rosenfeld R. Growth factors as therapeutic agents. J Bone Joint Surg 1996; 78A:1272-1286
54. Tremollieres FA, Strong DD, Baylink DJ, Mohan S. Insulin-like growth factor II and transforming growth factor beta 1 regulate insulin-like growth factor I secretion in mouse bone cells. Acta Endocrinol 1991; 125: 538-546
55. Urist MR. Bone: formation by autoinduction. Science 1965; 150:893-899
56. Wilton P. Treatment with recombinant human insulin-like growth factor I of children with growth hormone receptor deficiency (Laron syndrome). Kabi Pharmacia Study Group on insulin-like growth factor I treatment in growth hormone insensitivity syndromes. Acta Paediatr Suppl 1992; 383:137-142
57. Yaeger PC, Masi TL, de Oritz JL, Binette F, Tubo R, Mc Pherson J. Synergistic action of transforming growth factor-beta and insulin-like growth factor-I induces expression of Type II collagen and aggrecan genes in adult human articular chondrocytes. Exp Cell Res 1997; 15: 318-325
58. Yamada Y, Harada A, Hosoi T, Miyauchi A, Ikeda K, Otha H, Shiraki M. Association of transforming growth factor beta 1 genotype with therapeutic response to active vitamin D for postmenopausal osteoporosis. J Bone Miner Res 2000; 15:415-420
59. Yeh LC, Adamo ML, Olson MS, Lee JC. Osteogenic protein-1 and insulin-like growth factor I synergistically stimulate rat osteoblastic cell differentiation proliferation. Endocrinology 1997; 138: 4181-4190
60. Zdeblick TS, Ganayem AJ, Rapoff AJ, Swain C, Bassett T, Cooke ME, Markel M. Cervical interbody fusion cages. An animal model with and without bone morphogenetic protein. Spine 1998; 23:758-765
61. Zegzula HD, Buck DC, Brekke J, Wozney JM, Hollinger JO. Bone formation with use of rhBMP-2 (recombinant human bone morphogenetic protein-2). J Bone Joint Surg Am 1997; 79:1778-1790

Tables

Table 1: After 12 weeks quantitative computed tomographic analysis (QCT) was performed to measure bone mineral density (BMD), bone mineral content (BMC) and bony callus volume (BCV). [a] $p<0.05$ in comparison to group 1; [b] $p<0.05$ in comparison to group 2;

QCT (parameters)	Group 1 (n=8) Cage + PDLLA	Group 2 (n=8) Cage + PDLLA + IGF-I (0.5%) +TGF-ß1 (2.5%)	Group 3 (n=8) Cage + PDLLA + IGF-I (1%) +TGF-ß1 (5%)	Group 4 (n=8) Cage + PDLLA + IGF-I (2%) +TGF-ß1 (10%)
BMD (g/cm^3)	0.62+/-0.03 (0.59-0.66)	0,62 +/- 0.03 (0.58-0.66)	0.64 +/- 0.04 (0.59-0.68)	0.65 +/- 0.04 (0.60-0.69)
BMC (g)	2.1 +/-0.5 (1.3-2.6)	2.6 +/- 1.5 (2.7-5.8)	3.4 +/- 1.6 [a] (2.4-5.5)	3.5 +/- 1.6 [a] (2.4-5.5)
BCV (cm^3)	3.4 +/-0.9 (2.5-4.3)	4,2 +/- 1.1 [a] (4.4-7.1)	5.2 +/- 1.2 [a,b] (4.1-6.8)	5.4 +/- 1.0 [a,b] (4.1-6.8)

Table 2: Biomechanical analysis: After 12 weeks biomechanical analysis was performed to measure range of motion (ROM), neutral (NZ) and elastic zone (EZ) for the different test modes. [a] $p<0.05$ in comparison to group 1; [b] $p<0.05$ in comparison to group 2;

Test modes (degrees)	Group 1 (n=8) Cage + PDLLA	Group 2 (n=8) Cage + PDLLA + IGF-I (0.5%) +TGF-ß1 (2.5%)	Group 3 (n=8) Cage + PDLLA + IGF-I (1%) +TGF-ß1 (5%)	Group 4 (n=8) Cage + PDLLA + IGF-I (2%) +TGF-ß1 (10%)
Flexion				
ROM	3.0 +/- 0.9	2.9 +/- 1.0	2.9 +/- 1.2	2.7 +/- 1.0
NZ	0.5 +/- 0.7	0.5 +/- 0.7	0.7 +/- 0.7	0.6 +/- 0.5
EZ	2.5 +/- 0.9	2.4 +/- 0.9	2.3 +/- 0.8	2.1 +/- 0.8
Extension				
ROM	3.3 +/- 1.2	3.0 +/- 1.1	2.2 +/- 0.7 [a]	2.2 +/- 0.8 [a]
NZ	1.4 +/- 1.0	1.1 +/- 0.6	0.6 +/- 0.5	0.5 +/- 0.6
EZ	1.9 +/- 0.3	1.9 +/- 0.6	1.6 +/- 0.3	1.7 +/- 0.5
right rotation				
ROM	2.4 +/- 0.8	2.0 +/- 0.3	1.4 +/- 0.3 [a,b]	1.2 +/- 0.4 [a,b]
NZ	0.4 +/- 0.3	0.4 +/- 0.3	0.2 +/- 0.1 [a,b]	0.2 +/- 0.1 [a,b]
EZ	2.0 +/- 0.6	1.6 +/- 0.3	1.2 +/- 0.3 [a,b]	1.0 +/- 0.3 [a,b]
left rotation				
ROM	2.3 +/- 0.5	1.9 +/- 0.5	1.5 +/- 0.4 [a,b]	1.2 +/- 0.4 [a,b]
NZ	0.3 +/- 0.1	0.3 +/- 0.1	0.2 +/- 0.1 [a,b]	0.2 +/- 0.1 [a,b]
EZ	2.0 +/- 0.5	1.8 +/- 0.5	1.3 +/- 0.3 [a,b]	1.0 +/- 0.4 [a,b]
right bending				
ROM	4.1 +/- 1.0	3.2 +/- 0.7	2.5 +/- 0.7 [a,b]	2.4 +/- 0.6 [a,b]
NZ	0.7 +/- 0.4	0.6 +/- 0.3	0.6 +/- 0.3 [a]	0.5 +/- 0.3 [a]
EZ	3.4 +/- 0.9	2.8 +/- 0.8	1.9 +/- 0.7 [a]	1.9 +/- 0.6 [a]
left bending				
ROM	4.2 +/- 0.7	3.3 +/- 0.6	2.5 +/- 0.8 [a,b]	2.3 +/- 0.7 [a,b]
NZ	0.8 +/- 0.7	0.8 +/- 0.3	0.5 +/- 0.4 [a]	0.4 +/- 0.2 [a]
EZ	3.4 +/- 0.8	2.5 +/- 0.5	2.0 +/- 0.5 [a]	1.9 +/- 0.7 [a]

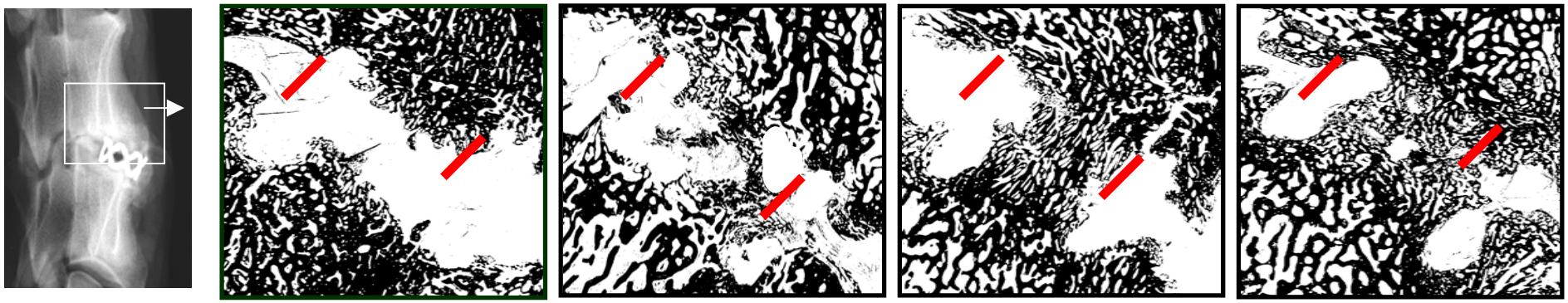
Table 3: After 12 weeks histomorphometrical analysis was performed and the following structural indices were calculated in the region of interest (ROI): sagittal diameter distance (SDD, baseline), bone volume / total volume (BV/TV), cartilage volume / total volume (CV/TV), mineralised cartilage volume / cartilage volume (mCV/CV). [a] $p<0.05$ in comparison to group 1; [b] $p<0.05$ in comparison to group 2;

Indices	Group 1 (n=8) Cage + PDLLA	Group 2 (n=8) Cage + PDLLA + IGF-I (0.5%) +TGF-ß1 (2.5%)	Group 3 (n=8) Cage + PDLLA + IGF-I (1%) +TGF-ß1 (5%)	Group 4 (n=8) Cage + PDLLA + IGF-I (2%) +TGF-ß1 (10%)
SDD (mm)	25.9 +/- 1.6 (24.3-27.8)	25.7 +/- 1.4 (24.0-27.1)	26.6 +/- 1.1 (25.3-28.3)	26.1 +/- 1.3 (24.6-28.1)
BV/TV (%)	41.8 +/-3.2 (31.7-50.2)	42.1 +/-2.4 (33.7-46.3)	43.3 +/-2.8 [a,b] (33.8-51.4)	43.6 +/-2.8 [a,b] (33.8-51.4)
CV/TV (%)	4.4 +/- 2.1 (1.9-9.2)	4.3 +/- 1.8 (2.1-8.0)	4.8 +/- 2.4 (0.8-14.6)	4.7 +/- 2.2 (1.3-11.2)
mCV/CV (%)	3.6 +/- 1.0 (1.9-5.8)	3.0 +/- 1.1 (1.4-5.5)	3.2 +/- 1.2 (1.8-5.7)	3.7 +/- 1.1 (2.0-5.4)

Table 4: After 12 weeks flurochrome analysis was performed. Depicted are the number of fusion sites (of the different groups at different time points) in which the flurochrome marker was present adjacent or within the cage or bone graft, respectively.

Indices	Group 1 (n=8) Cage + PDLLA		Group 2 (n=8) Cage + PDLLA + IGF-I (0.5%) +TGF-ß1(2.5%)		Group 3 (n=8) Cage + PDLLA + IGF-I (1%) +TGF-ß1 (5%)		Group 4 (n=8) Cage + PDLLA + IGF-I (2%) + TGF-ß1 (10%)	
	adjacent	within	adjacent	within	adjacent	within	adjacent	within
3 weeks	0	0	2	2	2	2	2	3
6 weeks	3	6	3	4	4	4	4	5
9 weeks	5	6	5	5	6	6	6	7

Figure 1a-d: Histomorphological analysis of the intervertebral fusion area: After 12 weeks interbody fusion was evaluated histomorphologically and histomorphometrically on midsagittal slides. Depicted is a digitalized view of a Safranin-O/ van Kossa staining of the intervertebral fusion area (magnification = 1.6x). Red bar represents the location of the branches of the cage. Lateral-X-ray of the sheep motion segment C3/4 allows orientation.



Anhand dieser Untersuchung konnte gezeigt werden, dass die kombinierte lokale Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage in der Lage ist, die intervertebrale Spondylodese zu stimulieren. Zusätzlich konnte eine geeignete Dosis dieser Wachstumsfaktorenkombination für die weiteren Untersuchungen definiert werden.

2.3.3 IGF-I und TGF-β1 im Vergleich zu autologem Knochenmaterial und BMP-2

Eine neues experimentelles Verfahren, wie die kombinierte lokale Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage, muss hinsichtlich seiner Effektivität an Standard-Therapieverfahren gemessen werden. Als Standard-Therapieverfahren zur ventralen interkorporellen Spondylodese der Halswirbelsäule werden die Stabilisierung mit trikortikalem Beckenkammspan und mittels Spongiosa-augmentierter intervertebraler Cages angesehen. Zusätzlich hat sich die Applikation des Wachstumsfaktors BMP-2 zur Stimulation der intervertebralen Spondylodese als „experimenteller goldener Standard“ etabliert.

- Daher sollte in diesen Untersuchungen die kombinierte lokale Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage mit der intervertebralen Spondylodese durch trikortikalen Beckenkammspan verglichen werden.
- Zusätzlich sollte in diesen Untersuchungen die kombinierte lokale Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage mit der intervertebralen Spondylodese durch Spongiosa-augmentierte intervertebrale Cages verglichen werden.
- Schließlich sollte in diesen Untersuchungen die kombinierte lokale Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage mit der intervertebralen Spondylodese durch BMP-2 verglichen werden.

SPINE Volume 27, Number 16, pp 1710-1723

IGF-I and TGF-β1 Application by a Poly-(D,L-Lactide)-Coated Cage Promotes Intervertebral Bone Matrix Formation in the Sheep Cervical Spine

Frank Kandziora, MD; Gerhard Schmidmaier, MD; Georg Schollmeier, MD; Herman Bail, MD; Robert Pflugmacher, MD; Thomas Görke, MD; Martin Wagner, MD; Michael Raschke, MD; Thomas Mittlmeier, MD; Norber P. Haas, MD

Study Design. A sheep cervical spine interbody fusion model was used to determine the effect of combined insulin-like growth factor-I (IGF-I) and transforming growth factor-beta-1 (TGF-β1) applied by a poly-(D,L-lactide) (PDLLA)-coated cage.

Objectives. The purpose of this study was to determine the effect of a new PDLLA carrier system, and to evaluate the effect of combined IGF-I and TGF-ß1 application in a sheep cervical spine model.

Summary and Background Data. Growth factors such as bone morphogenic protein-2 have been shown to promote spine fusion and to overcome the disadvantages of an autologous bone graft. The optimum growth factor for promoting spinal fusion and the optimum method for delivering such growth factors are still a matter of discussion.

Method. In this study, 32 sheep underwent C3–C4 discectomy and fusion: Group 1 (autologous tricortical iliac crest bone graft; n = 8), Group 2 (titanium cage; n = 8), Group 3 (titanium cage coated with a PDLLA carrier; n = 8), and Group 4 (titanium cage coated with a PDLLA carrier including IGF-I [5% w/w] and TGF-ß1 [1% w/w; n = 8). Blood samples, body weight, and body temperature were analyzed. Radiographic scans were performed before and after surgery, then at 1, 2, 4, 8, and 12 weeks, respectively. At the same time points, the disc space height, intervertebral angle, and lordosis angle were measured. After 12 weeks, the animals were killed, and fusion sites were evaluated using functional radiographic views of the animals in flexion and extension. Quantitative computed tomographic scans were performed to assess bone mineral density, bone mineral content, and bony callus volume. Biomechanical testing of the motion segment C3–C4 was performed in flexion, extension, axial rotation, and lateral bending. The stiffness, range of motion, neutral zone, and elastic zone were determined. Histomorphologic and histomorphometric analysis was performed, and polychrome sequential labelling was used to determine the time frame of new bone formation.

Results. There were no differences between the groups in terms of blood counts, body weight, and temperature. Over a 12-week period, cage Groups 2 to 4 showed significantly higher values for the intervertebral angle than for the bone graft. Functional radiographic assessment showed significantly lower residual flexion–extension movement in Group 4 than in any other group. The PDLLA-coated cages with IGF-I and TGF-β1 showed significantly higher values for bone mineral density, bone mineral content, and bony callus volume. The average stiffness in rotation and bending was significantly higher, and the range of motion, neutral zone, and elastic zone in rotation were significantly lower in Group 4 than in any other group. Although only one animal in Group 4 demonstrated solid bony fusion after 12 weeks, histomorphometric evaluation showed a more progressed bone matrix formation in the group that had PDLLA-coated cages with IGF-I and TGF-β1 than in any other group. Polychrome sequential labeling showed accelerated intervertebral bone matrix formation in Group 4.

Conclusions. The findings showed that PDLLA coating of cervical spine interbody fusion cages as a delivery system for growth factors was effective. Although IGF-I and TGF-β1 application by a PDLLA-coated interbody cage was not able to achieve solid bony fusion during the 12-week follow-up period, these growth factors significantly increased the results of interbody bone matrix formation. Additional longer-term studies are required to determine whether combined IGF-I and TGF-ß1 application leads to a successful spinal fusion. (Key words**:** animal model interbody fusion; biodegradable; cervical spine; IGF-I; poly(D-L-lactide); sheep; TGF-β1 growth factor) **Spine 2002;27:1710–1723**

From Unfall- und Wiederherstellungschirurgie, Universitätsklinikum Charité der Humboldt Universität Berlin, Campus Virchow Klinikum, Berlin, Germany

Supportet by MBI-Max-Biedermann Institut der Steinbeis Stiftung (RS/KF-82042)
Acknowledgement date: January 2, 2001
Second revision date: May 10, 2001
Second revision date: November 26, 2001
Acceptance date, February 12, 2002.
Device status/Drug statement: The device and drugs are the subject of this manuscript are not FDA approved for this indication, and are not commercially available in the United States.
Conflict interest: Institutional and foundation funds were received to support this work. No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.
DOI: 10.1097/01.BRS.0000020306.86675.60

Growth factors such as BMP-2 have been shown to accelerate spine fusion and overcome the disadvantages of an autologous bone graft.[3,4,7,8,12,18,30,36,48,49] The optimum growth factor for promoting spinal fusion and the optimum method for delivering such growth factors is still a matter of discussion.

Recently, *in vitro* and *in vivo* studies have demonstrated an osteoinductive effect of IGF-I and TGF-β1.[26,32,46] Whereas IGF-I stimulates the replication of osteoblasts and the synthesis of bone matrix,[14] TGF-β1 regulates different cell types directly involved in bone remodeling and bone matrix formation such as mesenchymal cells, chondrocytes, osteoblasts, and osteoclasts.[34,35]

In vivo studies have shown that decreased levels of IGF-I and TGF-β1 are associated with bone loss and osteoporosis,[1,7] whereas the local application of IGF-I and TGF-β1 can positively influence fracture healing.[17,33,41] A human study showed the positive influence of IGF-I on bone metabolism.[47] The systemic application of TGF-β1 stimulated osteoblast proliferation and increased bone matrix formation and bone remodeling in the bone defects of rabbits.[2] Locally applied TGF-β1 accelerated fracture healing in a dosage-dependent manner.[27] The combined application of IGF-I and TGF-β1 had a significantly higher stimulating effect on bone matrix formation in rat tibia fractures than the single application of IGF-I or TGF-β1.[34,38] The effects of isolated or combined application of IGF-I and TGF-β1 on spinal fusion are unknown.

Because growth factors are not specific for particular tissue, they have shown various effects on cellular processes. The effects of growth factors might be dosage dependent and could have negative effects at high or low doses.[9,43,47] Therefore, the local and controlled application of these factors seems to be necessary for stimulation of spinal fusion. Currently, especially collagen sponge carrier is in use for local application of growth factors in experimental spine fusion. However, the safety and accuracy of these carriers are questionable.[29,40,42]

Coating of implants with locally active growth factors could influence concepts of spine fusion. However, the local application of these factors at the place of requirement in desired concentrations still is an unsolved problem. A continuous drug release with high local but low systemic concentrations could be achieved by a biodegradable drug carrier. Recently, a biodegradable local drug delivery system enabling the "cold coating" of implants with potential thermolabile proteins such as growth factors has been described.[13,38,39] Various experiments have been performed to test the properties of this 10- to 14-μm thin poly-(D,L lactide) coating of implants.[39] Intramedullary implantation and extraction in rat tibias have indicated that the coating has high mechanical stability by demonstrating a loss of the coating from steel and titanium implants of less than 5%. The biodegradable carrier system depicted a constant reduction of 10% after 9 weeks *in vitro*. Growth factors incorporated in a biologically active form demonstrated less than a 5% loss of activity after storage for 1 year. *In vitro* and *in vivo* experiments indicated an 80% controlled release of growth factors from coated implants within 6 weeks after an initial peak.

Besides this, previous studies of rat tibia fractures showed that the poly-(D,L-lactide) (PDLLA) carrier itself applied *via* a intramedullary titanium implant had a significantly positive effect on bone matrix formation. As compared with the noncoated implant, the PDLLA-coated implant showed a significantly increased fracture consolidation rate in radiographic, biomechanical, and histomorphometric evaluation.[38]

On the basis of these studies, this new coating technique could be very promising in the field of spine surgery because it may be able to combine biomechanically well-established implants with biologically active substances. In this new combination, the implant serves as a device for spine fixation and as a local drug delivery system to improve spine fusion not only by the growth factors, but eventually also by the PDLLA carrier system itself.

For the clinical use of a carrier system or a growth factor, determination of its safety is a prerequisite. The safety of a carrier system and growth factor or growth factor combination can be assumed if there no side effects are associated with its use.

The purpose of this study was to determine the effect of a biodegradable PDLLA carrier system, and to evaluate the effect of combined IGF-I and TGF-β1 applied in an *in vivo* sheep cervical spine interbody fusion model. The relevant hypotheses of this study were as follows: 1) The PDLLA-coating of cages results in an effective carrier system for the local delivery of growth factors; 2) the PDLLA-coating of interbody fusion cages has a positive effect on interbody bone matrix formation; 3) combined IGF-I and TGF-β1 application significantly accelerates intervertebral bone matrix formation, as compared with the "spontaneous bone matrix formation potential"; and 4) combined IGF-I and TGF-β1 application *via* a PDLLA-coated cage is more effective than a tricortical iliac crest bone graft in cervical spine interbody fusion.

Materials and Methods

Study Design. For this study, 34 adult female merino sheep (2 years old) underwent C3–C4 discectomy and fusion. Two of the sheep were lost to follow-up evaluation. The remaining 32 sheep were assigned randomly to the following groups and treatments: Group 1 (autologous tricortical iliac crest bone graft; n = 8), Group 2 (meshed titanium cage; n = 8), Group 3 (meshed titanium cage coated with a biodegradable PDLLA carrier; n = 8), and Group 4 (meshed titanium cage coated with a biodegradable PDLLA carrier including IGF-I [5% w/w] and TGF-β1 [1% w/w]; n = 8).

After 12 weeks, all the sheep were killed, after which radiographic, biomechanical, and histologic evaluations were performed. All the animal experimental work was approved by local authorities.

Coating of the Cages. As a drug carrier system, PDLLA (Boeringer, Ingelheim, Germany) with a molecular weight of 30,000 Daltons was chosen. The meshed titanium cages of Groups 3 and 4 were immersed in the coating solution. In Group 4, recombinant human insulin-like growth factor-I (IGF-I; R&D Systems) (5% w/w) and recombinant human transforming growth factor-beta-1 (TGF-β1; R&D Systems) (1% w/w) were incorporated into the PDLLA solution. The average PDLLA coating mass of the cages was 3.02 ± 0.12 mg. Therefore, approximately 150 μg (5% w/w) of IGF-I and 30 μg (1% w/w) of TGF-β1 were incorporated into the coating.

Surgical Technique and Postoperative Care. All the sheep received 2 g of amoxicillin intravenously (intravenous Augmentan; SmithKline Beecham Pharma)

before surgery. The animals underwent the surgical procedure under general endotracheal anesthesia. For induction of anesthesia, thiopental-natrium 0.5 g (Trapanal; Byk Gulden) and fentanyldihydrogencitrat 0.1 mg (Fentanyl-Janssen; Janssen-Cilag) were used. For maintenance of anesthesia, inhalation of isofluran (Isofluran-Lilly; Lilly) and intravenous dosages of fentanyldihydrogencitrat 0.2 mg (Fentanyl-Janssen; Janssen-Cilag) were applied. The anterior part of the neck and the left iliac crest in Group 1 were prepared in a sterile fashion, and a left anterolateral approach to the cervical spine through a longitudinal skin incision was used. The longus colli muscle was incised in the midline, and the intervertebral disc C3–C4 was exposed.

After distraction of the motion segment with a Caspar distractor, anterior discectomy C3–C4 was performed. The endplates were shaved with a 2-mm high-speed diamond drill down to bleeding bone. No attempt was made to excise the posterior longitudinal ligament or expose the spinal canal. For interbody stabilization, meshed titanium cages (Motech GmbH, Schwenningen, Germany) 8 mm in height and 14 mm in diameter were used in Groups 2 to 4. In Group 1, an autologous bone graft with a height of 8 mm, a depth of 14 mm in depth, and an average width of 11 mm was taken from the left iliac crest. Before insertion, the volume of the bone graft was determined using the water displacement technique (Archimedes principle). The tricortical bone graft was inserted press-fit into the intervertebral space with the cortical shape of the graft anterior using Robinson's technique. Finally, the wound was irrigated with saline and the longus coli muscle closed with a running suture. The subcutaneous tissue and the skin were reapproximated with interrupted sutures, and a soft bandage was applied to the neck.

After surgery, the animals were maintained under observation until they had fully recovered from general anesthesia. They received two doses of metamizol-natrium 0.5 g (Novaminsulfon; Lichtenstein) per day intramuscularly for 5 days. Clinical examination was performed daily for the first 10 days, then weekly. The sheep were allowed *ad libitum* activity for the remainder of the experiment. Fluorochrome sequential labels administered intravenously 3, 6, and 9 weeks after surgery consisted of oxytetracycline (25 mg/kg) at 3 weeks, calcein green (15 mg/kg) at 6 weeks, and xylenol orange (90 mg/kg) at 9 weeks. The animals were killed 12 weeks postsurgery by an intravenous injection of potassium chloride after induction of anesthesia with thiopental-natrium 0.5 g (Trapanal; Byk Gulden) and fentanyldihydrogencitrat 0.1 mg (Fentanyl-Janssen; Janssen-Cilag). The complete cervical spine, including parts of the occiput and Th1, was then excised and cleaned from the surrounding tissue.

Blood and Serum Analysis. Blood and serum samples were taken from the saphenous vein of the sheep hind leg before and after surgery, then after 1, 2, 4, 8, and 12 weeks, respectively. The blood samples were analyzed for routine laboratory parameters: blood count, electrolytes, alkaline phosphatase, thyroid values, and glucose.

Body Weight and Temperature. Before surgery and after 1, 2, 4, 8, and 12 weeks, rectal body temperature and weight were determined.

Radiographic Analysis. To allow comparable radiographic evaluation, a special fixation device for the sheep cervical spine was developed. The reproducibility of the sheep cervical spine positioning in this fixation device was investigated by repeated measurements.Before surgery, one sheep in each group was selected randomly to have 10 lateral and 10 posteroanterior digital radiographic scans. Between the radiographic scans, the sheep was removed from the fixation device, turned around, and then refixed in the fixation device. Subsequently, the complete series of linear and angular measurements was performed on each radiographic scan.

Lateral and posteroanterior digital radiographic scans (radiograph unit: Mobilett Plus; Siemens AG, Germany; radiograph films: Fuji CR 24 × 30; Fuji, Germany) were performed before and after surgery, then after 1, 2, 4, 8, and 12 weeks, respectively. During the same periods, the anterior, middle, and posterior intervertebral disc space heights (DSH), intervertebral angle (IVA), and lordosis angle (LA) of the motion segment C3–C4 were measured on lateral radiographic scans (Figure 1). Average intervertebral DSH was calculated from anterior, middle, and posterior DSH measurements (anterior, middle, and posterior DSH/3). All radiographic measurements were evaluated by three independent observers.

Functional Radiographic Analysis. After the animals were killed, fusion sites were evaluated using lateral digital functional radiographic scans in flexion and extension (Figure 2) (radiograph unit: Mobilett Plus; Siemens AG, Germany; radiograph films: Fuji CR 24 × 30; Fuji, Germany). For this purpose, Th1 was rigidly fixed with a Steinmann pin while a 60-N load was applied through C1 using a newton meter (Inha GmbH). Flexion–extension differences of intervertebral angle (IVA) and lordosis angle (LA) were calculated. All functional radiographic measurements were evaluated by three independent observers.

Quantitative Computed Tomographic Analysis. Quantitative computed tomographic scans (QCT) were performed using a Siemens Somatom plus 4 scanner (Siemens, Erlangen, Germany). Axial cuts with a 1-mm slice thickness were made parallel to the intervertebral disc space. Bone mineral density (BMD) measurements of the callus have been described in detail previously.[20] Measurements were calibrated with a 6-point bone mineral density phantom and performed using specific software of the scanner (Sienet Magic View VA 30A; Siemens). Bony callus volume (BCV) was measured using an image analyzing system (Zeiss KS 400; Zeiss GmbH, Germany). Bone mineral content (BMC) was calculated from BMD and BCV measurements (BMC = BCV × BMD). All radiographic CT measurements were evaluated by three independent observers.

Biomechanical Analysis. After the animals were killed, biomechanical testing was performed by a nondestructive flexibility method using a nonconstrained testing apparatus described in detail previously.[19,20] Pure bending moments of 6-Nm load were applied to the motion segments C3–C4 using a system of cables and pulleys to induce flexion, extension, left and right lateral bending, and left and right axial rotation. Tension was applied to the cables with an uniaxial testing machine (1456; Zwick GmbH, Ulm, Germany). Three-dimensional displacement of each motion segment was measured using an optical measurement system (Qualysis, Sävebalden, Sweden). Triangular markers with three diodes (Qualysis) were attached to the bodies of C3 and C4. Marker positions were detected with two cameras and recorded with a computerized motion analysis

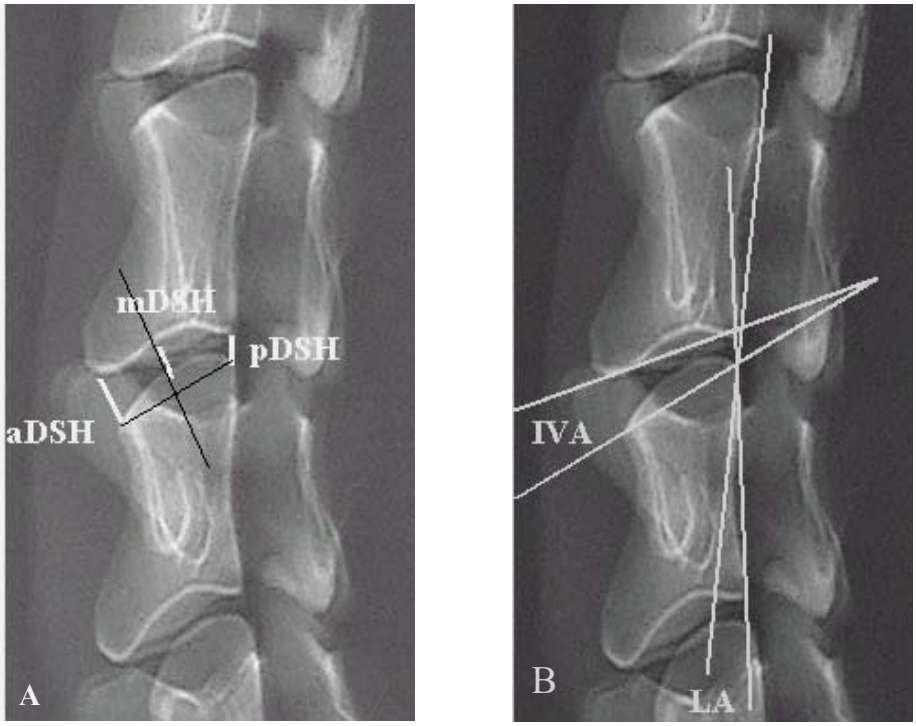


Figure 1. Radiographic measure-ments of anterior disc space height (aDSH), posterior disc space height (pDSH), middle disc space height (mDHS) **(A)**, intervertebral angle (IVA), and lordosis angel (LA) **(B)**.

system (PC-Reflex; Qualysis). Angular displacement of the upper vertebra (C3) in relation to the lower vertebra (C4) was calculated from the marker position using custom-made computer software. The experimental error associated with this method was ±0.1°.[21] The mean apparent stiffness values in the elastic zone were calculated from the corresponding load-displacement curves. Range of motion (ROM), neutral zone (NZ), and elastic zone (EZ) were determined.

Histomorpologic, Histomorphometric, and Fluorochrome Analysis. All C3–C4 motion segments were harvested at 12 weeks for bone histology. The motion segments had been fixed for 7 days in 10% normal buffered formaldehyde, followed by dehydration in ascending concentrations of ethanol, and embedded undecalcified in methylmethacrylate (Technovit 9100; Heraeus Kulzer GmbH, Germany).

For histomorphologic and histomorphometric analysis, longitudinal sections in the sagittal plane were cut at 6 m with a Leica SM 2500S microtome and a 40° stainless steel knife. Afterward, the residual parts of the cages were removed, and the following stains were used: safranin-O/lightgreen, safranin-O/v. Kossa, astrablue, and Masson-Goldner. Masson-Goldner stainings were used for histomorphologic analysis. Histomorphometric parameters were measured on the residual stainings using a Leica DM-RB microscope and the image analyzing system (Zeiss KS 400; Zeiss GmbH, Germany). Parameters were measured at a magnification of ×1.6.

Intervertebral fusion was categorized histologically according to Zdeblick et al.[49] The cage–bone interface and the tissue content inside the cages were analyzed using the following point system: cage–vertebral interface: empty (0 points), fibrous tissue (1 point), bone (2 points) and tissue inside cage: empty (0 points), fibrous cartilage (1 point), bone (2 points). Four points indicated that a successful arthrodesis or fusion had occurred. Three points were graded as a developing fusion.

The sagittal diameter distance (S) of C3 and the average preoperative DSH were determined to define the size of the region of interest (ROI) for histomorphometric evaluation (Figure 3). The complete intervertebral fusion area was included in this ROI. The following structural indexes were calculated in the ROI: bone volume–total volume (BV/TV), cartilage volume–total volume (CV/TV), and mineralized cartilage volume–cartilage volume (mCV/CV).

For flurocrome analysis, longitudinal sections in the parasagittal plane were cut at 400 µm with a precise macrogrinding machine (Fa. Exact, Norderstedt, Germany). These slices then were ground to a thickness of 80 µm using a precise microgrinding machine (Fa. Exact). Fluorocrome markers were analysed under appropriate lighting conditions using a Leica DM-RB microscope and an image analysing system (Zeiss KS 400; Zeiss GmbH). Parameters were measured at a magnification x1.6.

Fluorochrome analysis of intervertebral fusion areas has been described in detail previously.[50] The first appearance of the marker served to time formation of new bone matrix. The presence or absence of each marker around or within the cage or bone graft, respectively, was used to determine the relative time frame of new bone formation.

Statistical Analysis. Comparison of data was performed using one-way ANOVA for independent samples followed by Tukey *post hoc* analysis for multiple comparison procedures with Bonferroni correction for multiple measurements. Intraobserver variability for radiographic and functional radiographic evaluation and CT measurements was determined using kappa statistics. Statistically significant differences were defined at a 95% confidence level. The values are given as mean ± standard deviation. The statistical evaluation was supported by SPSS (release 7.0; SPSS, Chicago, IL) software.

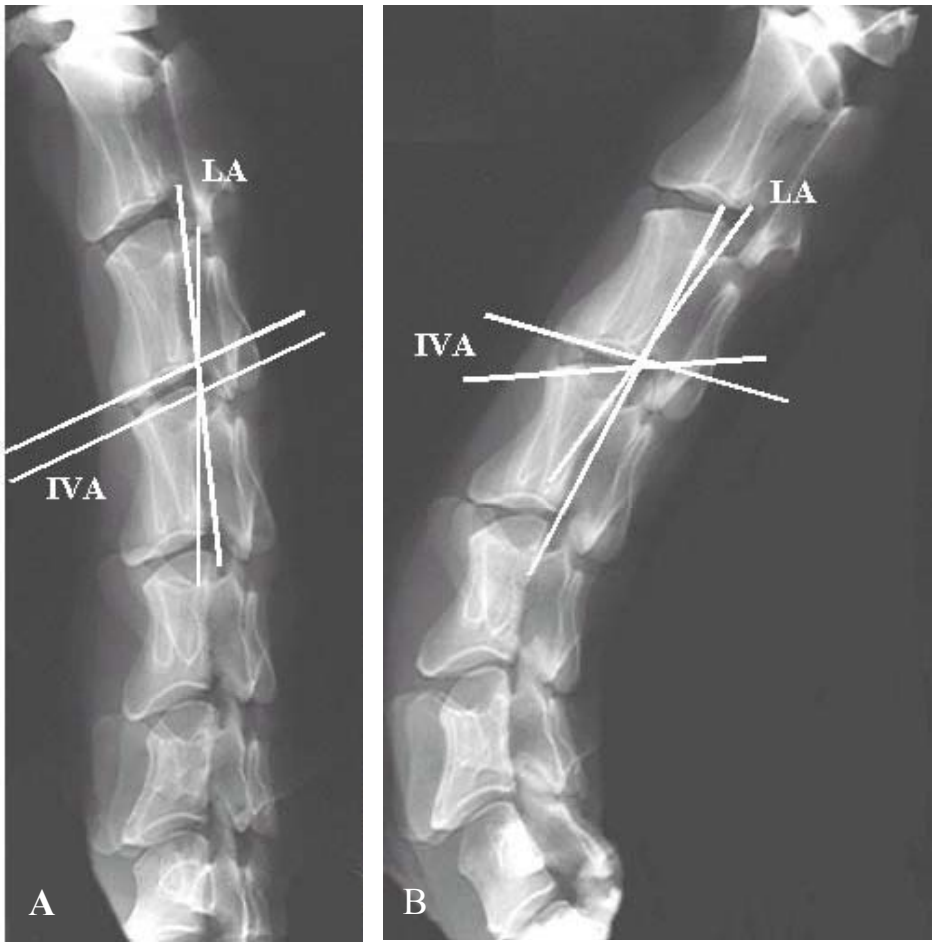


Figure 2. The motion segment C3 – C4 was evaluated using lateral digital functional radiographic scans in flexion **(A)** and extension **(B)**. Flexion-extension differences of intervertebral angle (IVA) and lordosis angle (LA) were calculated.

Results

Failure Parameters and Complications

One animal died of anesthesiologic complications on day 0. In Group 2, one animal had to be killed during week 6 because of a sore mouth (contagious ecthyma). Both animals were excluded from the study and replaced by other animals.

In Group 1, hematoma developed in animal 6 at the donor site of the iliac crest graft. In Group 2, superficial seroma developed in animal 4 at the neck. Both complications healed under conservative treatment without further difficulties.

Blood and Serum Analysis Results

A full blood count did not show any significant changes between the groups throughout the experiment. Even after 1 week, no significant changes were found for average hemoglobin, erythrocyte, and leukocyte levels.

The levels of electrolytes (Na, K, Cl, CA) did not show significant changes during the experimental period. Furthermore, no changes in thyroid hormones, alkaline phosphatase, or glucose levels were found throughout the observation period between any of the groups.

Body Weight and Temperatur

No significant differences were found between any of the groups in mean body temperature or weight throughout the experimental period. After surgery, a slight and constant increase in body weight of all the sheep in all the groups was determined. Except for animal 6 in Group 1, in which a hematoma developed at the iliac crest donor site and a slight increase of body temperature occurred during Weeks 1 and 2, none of the animals showed any indication for local or systemic infections throughout the observation time.

Radiographic Results

The reproducibility of sheep cervical spine positioning in the fixation device for radiographic evaluation was investigated by repeated measurements. Reproducibility of average DSH and IVA was high, showing a maximum difference on 10 radiographic scans of 1 mm (approximately 15% of total value) and 2° (approximately 15% of total value), respectively. However, reproducibility of LA was moderate, showing a maximum difference on 10 radiographic scans of 4.5° (approximately 65% of total value). Intraobserver agreement for radiographic measurements was good, with kappa values ranging between 0.77 and 0.96.

Preoperative baseline values for all the radiographic parameters showed no differences between the groups. No significant differences were found for average disc space height between any of the groups throughout the observation period (Figure 4A). At 4, 8, and 12 weeks, all

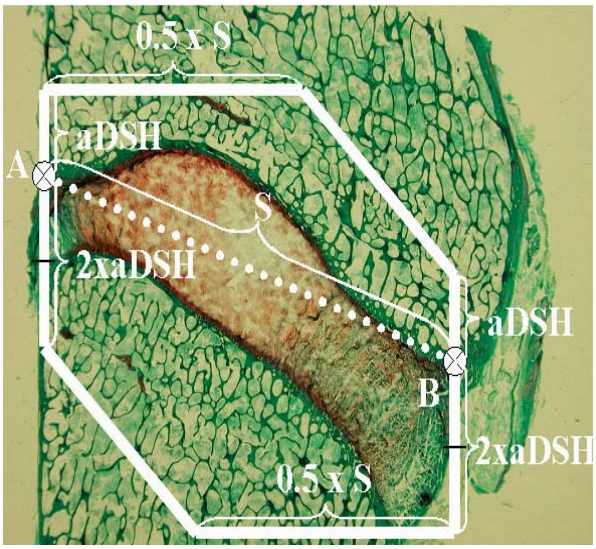


Figure 3. The region of interest (ROI) for histomorphometric evaluation was determined using the pints A (posterior edge of the endplate C3) and B (anterior edge of the endplate C3) and the preoperative average disc space height (aDSH) evaluated on lateral x-ray views of the motion segment C3–C4. The distance between A and B was defined as the sagittal diameter distance (S).

the cage groups (Groups 2 to 4) showed significantly higher values for IVA ($P < 0.05$; Figure 4B) than the autologous iliac crest graft group (Group 1). During the experimental period, no significant differences in the IVA were found between the cage groups. At 4, 8, and 12 weeks, the cage plus PDLLA plus IGF-I and TGF-β1 group (Group 4) showed significantly higher values for LA ($P < 0.05$; Figure 4C) than the autologous iliac crest graft group (Group 1). There were no differences in LA between the cage groups. Radiographic scans of the different groups after 12 weeks are depicted in Figure 5.

Functional Radiographic Results

Intraobserver agreement for functional radiographic measurements was excellent, showing kappa values ranging between 0.82 and 0.93. Flexion–extension measurements of LA did not show any difference between Groups 3 and 4 (Table 1). In addition, functional radiographic assessment showed significantly lower residual flexion–extension movement in the PDLLA-coated cages with IGF-I and TGF-β1 (Group 4) than in the other groups ($P < 0.05$).

Quantitative Computed Tomographic Results

Intraobserver agreement for CT measurements was excellent, showing kappa values ranging between 0.82 and 0.97. After 12 weeks, there were no significant differences between bone mineral content (BMC) and bony callus volume (BCV) among the iliac crest graft group (Group 1), the cage group (Group 2), and the cage plus PDLLA group (Group 3). In the cage plus PDLLA group (Group 3), bone mineral density of the callus (BMD) was significantly higher than in Groups 1 and 2 ($P < 0.05$). The PDLLA-coated cages with IGF-I and TGF-β1 (Group 4) showed significantly higher values for BCV ($P < 0.05$) than those in any other group (Figure 6, Table 2). Interestingly, there was no significant difference for BMD of the callus between the PDLLA-coated cages (Group 3) and the PDLLA-coated cages with IGF-I and TGF-β1 (Group 4).

Biomechanical Results

Biomechanical results for stiffness, range of motion (ROM), neutral zone (NZ), and elastic zone (EZ) are depicted in Figure 7 and Table 3. The highest stiffness values and the lowest ROM, NZ, and EZ values were constantly found for Group 4. The average stiffness in axial rotation and lateral bending was significantly higher ($P < 0.05$) in the group with PDLLA-coated cages with IGF-I TGF-β1 and than in any other group. In Group 4, ROM, NZ, and EZ in rotation and ROM during lateral bending were significantly lower ($P < 0.05$) than in any other group. Interestingly, after 12 weeks, there was a significantly lower axial rotation ROM in the coated (Group 3) and noncoated cage groups (Group 2) than in the bone graft group (Group 1).

Histomorphologic Results

Histomorphologic analysis supported the findings of the radiographic and biomechanical examinations (Figure

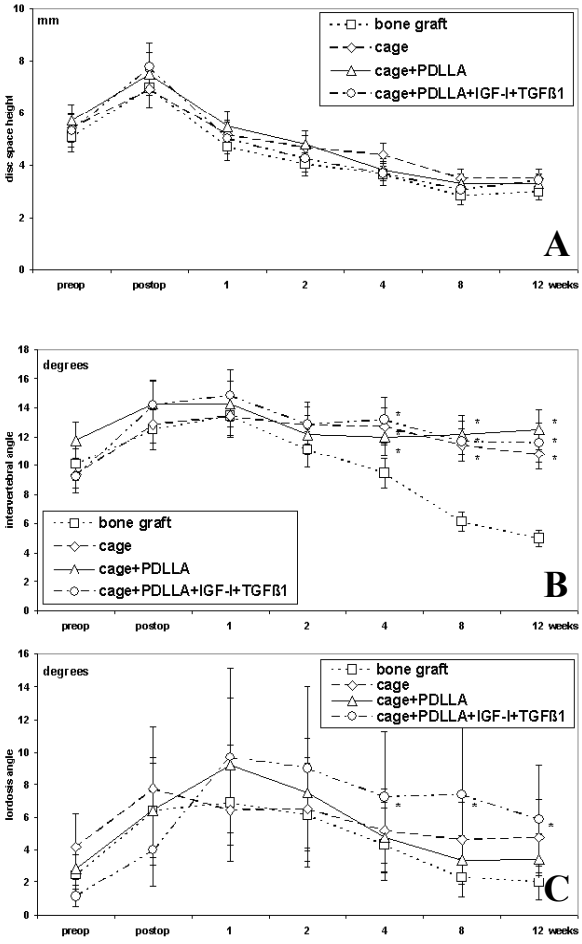
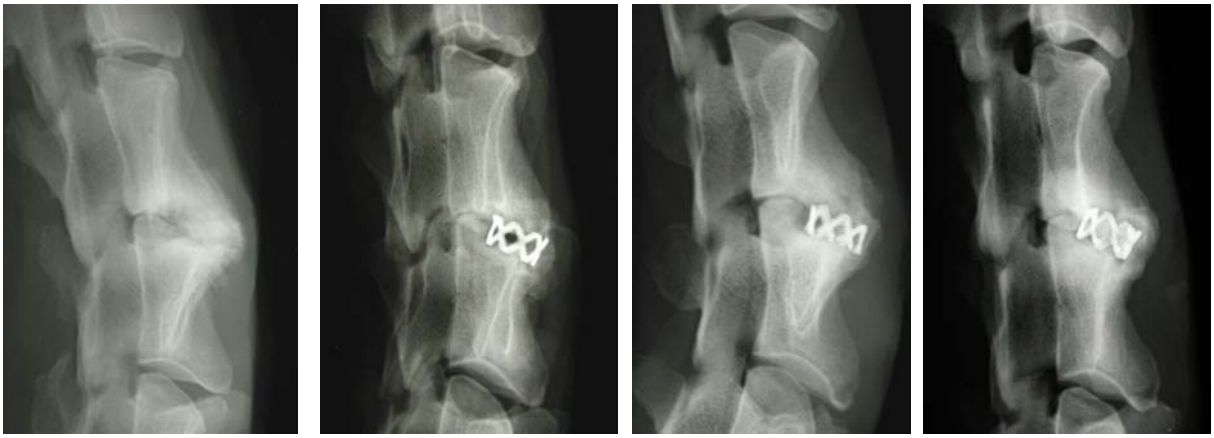


Figure 4. Radiographic analyses. **A–C,** Average disc space height, intervertebral angle, and lordosis angle of the different groups throughout the observation period. $P < 0.05$ in comparison with the bone graft.



Group 1
bone graft

Group 2
cage

Group 3
cage + PDLLA

Group 4
cage+PDLLA+IGF1+TGF-β1

Figure 5. Radiographic analyses. After 12 weeks, boney fusion was evaluated on radiographic scans (animal 8 of each group).

8). Group 1 (autologous iliac crest bone graft) showed extensive callus formation with cartilage and connective tissue cells. The bone graft was mainly absorbed. Osteoclastic activity was noted as an indication of graft resorption (Figure 8). Most of the callus was seen ventrally. In the noncoated (Group 2) and PDLLA-coated (Group 3) cage groups, mainly fibroblasts and occasionally cartilage cells were observed between the endplates (Figure 8), without major differences between the two groups. Minimal osteoclastic activity was observed at the endplates. Little capillary ingrowth was noted (Figure 8). The cages were surrounded by a distinct small line of fibroblasts. Group 4, stabilized by cages coated with PDLLA plus IGF-I and TGF-β1, showed bony islands between the endplates (Figure 8), with cartilage and fibrous tissue components. These findings were accompanied by capillary ingrowth and resorptive lacunas. The tissue surrounding the cages appeared similar to those in Groups 2 and 3. No differences in the amounts of macrophages were seen in the histologic sections of the different groups.

As compared with the other groups with IGF –I and TGF-β1 (Group 4) demonstrated slightly higher values in the histologic fusion score. No difference in fusion score values were found between the bone graft (Group 1) an cage alone (Group 2) or the cage plus PDLLA group (Group 3).

Histomorphometric Results.

The results of histomorphometric analysis are presented in Table 4. Histomorphometric analysis showed no significant differences of sagittal diameter index (baseline) between all groups. Compared to the other groups histomorphometric parameters revealed significantly progressed bone formation (BV/TV) in Group 4 ($P < 0.05$). There were no differences in histomorphometric parameters between Group 2 and 3, except for bone formation (BV/TV) ($P < 0.05$), which was higher in the PDLLA coated cages. Group 1 showed significantly higher histomorphometric values for CV/TV and mCV/TV than all other groups ($P < 0.05$).

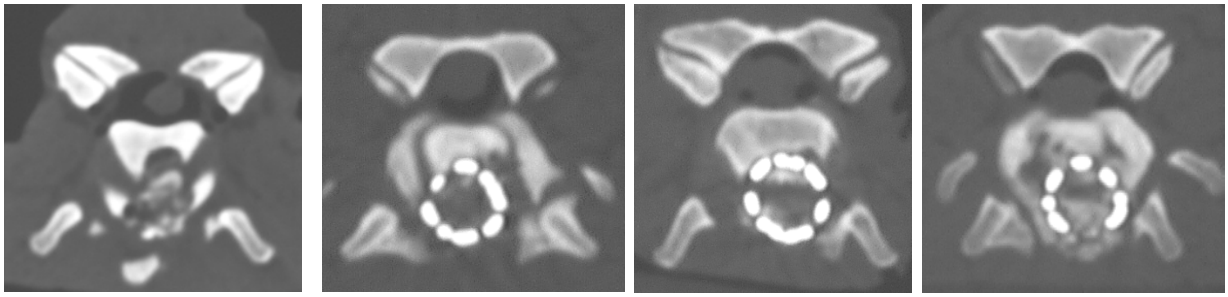
Fluorochrome Analysis Results.

The results of fluorochrome analysis are depicted in Table 5. The cages coated with IGF-I and TGF-β1 exhibited earlier new bone formation both within and around the cages than those in all other groups. There were no significant differences between Groups 2 and 3, except for new bone formation around the cages at 9 weeks, which was higher with the PDLLA-coated cages.

Table 1. After 12 Weeks, Functional Radiographic Evaluation in Flexion–Extension of the 4 Groups Was Determined: Flexion–Extension Differences of Intervertebral Angle (IVA) and Lordosis Angle (LA) Were Calculated

Flexion-Extension Difference in Degrees	Group 1 (n=8) Bone Graft	Group 1 (n=8) Cage	Group 1 (n=8) Cage + PDLLA	Group 1 (n=8) Cage + PDLLA + IGF-I + TGF-ß1
IVA	13.6 +/-3.7 (6.0-13.5)	8.6 +/- 2.6 (6.0-10.0)	8.7 +/-2.4 (7.0-10.5)	5.9 +/- 1.9 [a,b,c] (3.5-7.5)
LA	14.1 +/-5.0 (6.5-17.0)	8.6 +/- 3.1 (6.0-12.0)	7.9 +/-2.7 [a] (4.0-9.5)	6.3 +/- 1.6 [a,b] (5.0-8.0)

[a] p<0.05 in comparison to the bone graft (group 1)
[b] p<0.05 in comparison to the cage (group 2)
[c] p<0.05 in comparison to the PDLLA coated cage (group 3).



Group 1
bone graft

Group 2
cage

Group 3
cage + PDLLA

Group 4
cage+PDLLA+IGF1+TGF-ß1

Figure 6. CT analyses. After 12 weeks, interbody fusion was evaluated on axial CT scans parallel to the intervertebral space (animal 8 of each group).

Discussion

The objective of this study[1] was to determine the effect of a biodegradable PDLLA carrier system,[2] and to evaluate the effect of combined IGF-I and TGF-β1 applied in a *in vivo* sheep cervical spine interbody fusion model.

Biodegradable PDLLA Carrier System

The optimum method for delivering growth factors to the corresponding field still is a matter of discussion. For local application of growth factors in experimental spine fusion, especially the collagen sponge carrier is in use. Yet, the safety and accuracy of these carriers are questionable.[29,40,42] A fast and uncontrolled release of drugs from collagen sponges with potential unexpected tissue reactions to the drugs or the carrier system has been described.[40] An accurate intraoperative placement of these carriers in the desired region appears to be difficult, and inaccurate positioning may lead to uncontrolled interactions with the surrounding tissue. Martin et al,[29] for example, showed in an intertransverse spinal arthrodesis model that soft tissue compression of the collagen sponge carrier prevented bone induction at standard growth factor doses. Safety of the collagen sponge carrier also is questionable because it consists partially of bovine material that can lead to allergic reactions.[42] Additionally, recent controversies over BSE-transmission through bovine material also are a matter of safety discussion.

Various techniques for the coating of biomaterials and local drug release have been described. Poly-(lactic acid) (PLA) and poly-(glycolide acid) (PGA) or their copolymers have been widely used as biodegradable implants and drug delivery systems in orthopedic surgery.[25] The examined PDLLA degrades similarly to PGA and PLA by chemical, thermal, mechanical, and physical mechanisms,[11] whereas chemical degradation primarily through hydrolysis and metabolism in the citric acid cycle is the most important.[22,23] Many studies have investigated the biocompatibility of poly-(α-hydroxy acids) and the local tissue response of PLA and PGA or their copolymers. The tissue response depends on the amount and the degradation rate of the material.[6,15,22,28] Although mild inflammatory reactions have been observed when large amounts of polylactides are used, they generally are well tolerated.[16]

In a drug delivery system, the drug release kinetics of the carrier is of fundamental importance to obtain a therapeutic concentration in tissue and to avoid toxic side concentrations. Drugs can be released from polymers by diffusion and polymer swelling, followed by fast diffusion-controlled release and polymer erosion.[11] The drug release from the PDLLA used in the current study takes place in two steps, primarily by diffusion without mass loss, followed by erosion of the matrix.[43]

Table 2. After 12 Weeks, Quantitative Computed Tomographic Analysis (QCT) Was Performed to Measure Bone Mineral Density (BMD), Bone Mineral Content (BMC), and Bony Callus Volume (BCV)

QCT	Group 1 (n=8) Bone Graft	Group 1 (n=8) Cage	Group 1 (n=8) Cage + PDLLA	Group 1 (n=8) Cage + PDLLA + IGF-I + TGF-ß1
BMD(g/cm³)	0.58 +/-0.04 (0.64-0.56)	0.58 +/- 0.04 (0.65-0.56)	0.62+/-0.03 [a] (0.66-0.59)	0.65 +/- 0.04 [a,b] (0.68-0.59)
BMC (g)	2.3 +/-1.0 (1.7-3.1)	1.9 +/- 0.7 (1.2-2.8)	2.1 +/-0.5 (1.3-2.6)	3.4 +/- 1.6 [b,c] (2.4-5.5)
BCV (cm³)	4.0 +/-1.0* (3.8-5.1)	3.3 +/- 1.2 (2.0-4.7)	3.4 +/-0.9 (2.5-4.3)	5.2 +/- 1.2 [a,b,c] (4.1-6.8)

[a] p<0.05 in comparison to the bone graft (group 1)
[b] p<0.05 in comparison to the cage (group 2)
[c] p<0.05 in comparison to the PDLLA coated cage (group 3).
* Initial callus volume of the bone graft was 1.7 cm³

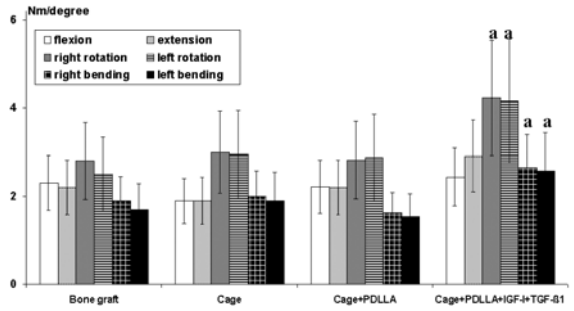


Figure 7. Biomechanical analysis. Stiffness of the different groups for the different test modes. [a] indicates P < 0.05 in comparison with the bone graft (Group 1), with the cage (Group 2), and with the PDLLA-coated cage (Group 3).

The degradation of copolymers depends on the amount of amorphous relative to crystalline polymer, with increased degradation found in amorphous regions.[5,31] Semicrystalline PLLA homopolymer degrades at a slower rate than the amorphous PDLLA homopolymer used in the current study. The rate of degradation also depends on the local environment. A well-vascularized environment leads to a more rapid degradation than occurs in avascular regions.[24]

The local application of bioactive molecules from biodegradable PLA or PGA systems were examined in several *in vitro* and *in vivo* studies. Gombotz et al[10] reported that the controlled release of TGF-β1 from a biodegradable poly-(D,L-lactide-coglycolide acid) supported bone ingrowth into the carrier system. Zegzula et al[50] demonstrated a dose-dependent healing of bone defects with rhBMP-2 from porous PDLLA implants in a rabbit model. Hermann et al[13] showed that incorporating antithrombotic drugs in a biodegradable PDLLA coating of coronary stents led to a significant reduction in local thrombotic reactions and restenosis of coronary vessels. The local application of recombinant human bone morphogenetic protein (rhBMP-2) from collagen sponges or bioabsorbable poly-(D,L-lactide-coglycolide) (PLA/PGA) beads with osteopromotive membranes to rat mandibular defects showed that PLA/PGA is superior to collagen sponge carrier for bone regeneration.[50]

In the current study, a biodegradable PDLLA carrier system was used for application of growth factors. Previous studies showed that during degradation of polymers, breakdown products are formed that may alter the physiologic environment.[25] Therefore, it might have been possible that these products influence healing processes. To examine the influence of the PDLLA on spinal fusion, PDLLA coated cages (Group 3) and noncoated cages (Group 2) were investigated. The blood, serum, body weight, and body temperature evaluations did not show any significant difference between the two groups. Additionally, histomorphologic evaluations indicated no voids in the interbody fusion mass or inflammatory reactions associated with the PDLLA coating. No differences in the amounts of macrophages between the two groups could be determined. Therefore, on the basis of these preliminary results, no

Table 3. Biomechanical Analysis: After 12 Weeks, Biomechanical Analysis Was Performed to Measure Range of Motion (ROM) and Neutral (NZ) and Elastic Zones (EZ) for the Different Test Modes

QCT	Group 1 (n=8) Bone Graft	Group 1 (n=8) Cage	Group 1 (n=8) Cage + PDLLA	Group 1 (n=8) Cage + PDLLA + IGF-I + TGF-β1
Flexion				
ROM	2.9 +/- 1.0	3.9 +/- 0.8	3.0 +/- 0.9	2.9 +/- 1.2
NZ	0.7 +/- 0.5	1.3 +/- 0.6	0.5 +/- 0.7	0.7 +/- 0.7
EZ	1.9 +/- 0.7	2.6 +/- 0.8	2.5 +/- 0.9	2.3 +/- 0.8
Extension				
ROM	3.3 +/- 1.5	3.9 +/- 1.3	3.3 +/- 1.2	2.2 +/- 0.7 [b]
NZ	0.9 +/- 0.7	1.0 +/- 0.6	1.4 +/- 1.0	0.6 +/- 0.5
EZ	2.4 +/- 0.8	2.9 +/- 0.9	1.9 +/- 0.3	1.6 +/- 0.3 [b]
Right Rotation				
ROM	3.3 +/- 1.4	2.3 +/- 1.0 [a]	2.4 +/- 0.8 [a]	1.4 +/- 0.3 [a,b,c]
NZ	0.7 +/- 0.3	0.5 +/- 0.1	0.4 +/- 0.3	0.2 +/- 0.1 [a,b,c]
EZ	2.7 +/- 1.1	2.0 +/- 0.9	2.0 +/- 0.6	1.2 +/- 0.3 [a,b,c]
Left Rotation				
ROM	3.3 +/- 1.4	2.3 +/- 0.8 [a]	2.3 +/- 0.5 [a]	1.5 +/- 0.4 [a,b,c]
NZ	0.7 +/- 0.5	0.4 +/- 0.1	0.3 +/- 0.1	0.2 +/- 0.1 [a,b,c]
EZ	2.7 +/- 1.8	1.9 +/- 0.7	2.0 +/- 0.5	1.3 +/- 0.3 [a,b,c]
Right bending				
ROM	4.5 +/- 2.4	3.8 +/- 1.0	4.1 +/- 1.0	2.5 +/- 0.8 [a,b,c]
NZ	1.5 +/- 1.5	1.0 +/- 0.4	0.7 +/- 0.4 [a]	0.6 +/- 0.3 [a,b]
EZ	3.0 +/-1.3	2.8 +/- 0.7	3.4 +/- 0.9	1.9 +/- 0.7 [b]
Left bending				
ROM	4.6 +/- 2.2	3.9 +/- 1.2	4.2 +/- 0.7	2.5 +/- 0.8 [a,b,c]
NZ	1.7 +/- 1.6	1.0 +/- 0.4	0.8 +/- 0.7 [a]	0.5 +/- 0.4 [a,b]
EZ	2.9 +/- 0.7	2.9 +/- 0.8	3.4 +/- 0.8	2.0 +/- 0.5 [b]

[a] p<0.05 in comparison to the bone graft (group 1)
[b] p<0.05 in comparison to the cage (group 2)
[c] p<0.05 in comparison to the PDLLA coated cage (group 3).

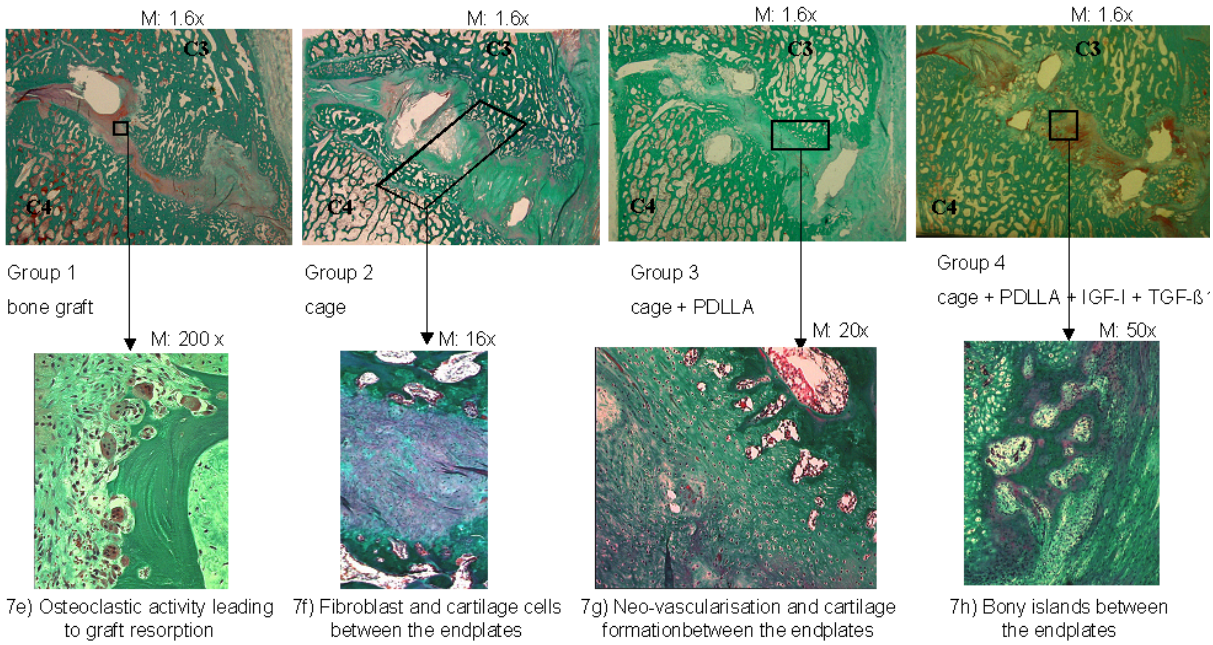


Figure 8. Histomorphologic analyses of the intervertebral fusion area. After 12 weeks, interbody fusion was evaluated histomorphologically and histomorphometrically (animal 8 of each group). M = magnification.

negative effects were associated with the use of the PDLLA carrier. However, in contrast to previous studies on rat tibia fractures,[38] an impressive positive effect of the PDLLA carrier on bone matrix formation could not be found. Yet, as compared with the noncoated cages, all the PDLLA-coated cages showed significantly higher values for the BMD of the callus and the ratio of bone volume to total volume; slightly higher values for functional radiographic evaluation of the lordosis angle, BMC, and BCV; lower values for ROM in flexion and extension; and higher values for new bone formation adjacent to the cage after 9 weeks. Therefore, the PDLLA carrier may have a positive effect on bone matrix formation, although it could not be detected in this study, probably because of the limited number of animals used.

IGF-I and TGF-ß1

For the first time, a combination of IGF-I and TGF-β1 was applied in a sheep cervical spine interbody fusion model. It was not the purpose of this study to achieve solid bony fusion. One criterion for the definition of bony fusion is the absence of motion in the fused motion segment. Therefore, it would not be useful to determine a residual range of motion or other biomechanical data used in a completely fused motion segment. Yet, these biomechanical data are crucial in such a study because they are highly precise. Therefore, aim of this study was to analyze a developing "bony fusion" at an early time (12 weeks) to determine subtle distinctions between the varying fusion techniques.

It is well known that simply the surgical intervention. including the decortication of the endplates, seems to stimulate intervertebral bone matrix formation to a limited degree.[37] To evaluate this "spontaneous bone matrix formation potential" with a growth factor–stimulated fusion, the cage-alone group (Group 2) was compared with the growth factor group (Group 4). The growth factor group (Group 4) showed a bone matrix formation potential significantly higher than the "spontaneous bone matrix formation potential" of the cage-alone group (Group 2).

Table 4. After 12 Weeks, Histomorphometric Analysis Was Performed and the Following Structural Indices Were Calculated in the Region of Interest (ROI): Sagittal Diameter Distance (SDD, Baseline), Bone Volume/Total Volume (BV/TV), Cartilage Volume/Total Volume (CV/TV), Mineralized Cartilage Volume/Cartilage Volume mmCV/CV)

Indices	Group 1 (n=8) Bone Graft	Group 1 (n=8) Cage	Group 1 (n=8) Cage + PDLLA	Group 1 (n=8) Cage + PDLLA + IGF-I + TGF-β1
SDD(mm)	26.2 +/- 1.0 (24.0-27.3)	25.6 +/- 1.2 (24.6-27.0)	25.9 +/- 1.6 (24.3-27.8)	26.6 +/- 1.1 (25.3-28.3)
BV/TV(%)	31.4 +/- 3.9 (24.8-39.0)	38.3 +/- 4.1[a] (26.4-52.1)	41.8+/-3.2[a,b] (31.7-50.2)	43.3+/-2.8[a,b,c](33.8-51.4)
CV/TV(%)	10.1+/- 2.8 (1.0-22.1)	4.3 +/- 2.4[a] (1.4-11.0)	4.4 +/- 2.1[a] (1.9-9.2)	4.8 +/- 2.4[a] (0.8-14.6)
mCV/CV(%)	5.5 +/-2.0 (0.6-9.4)	3.4 +/- 1.8[a] (0.8-5.0)	3.6 +/- 1.0[a] (1.9-5.8)	3.2 +/- 1.2[a] (1.8-5.7)

[a] p<0.05 in comparison to the bone graft (group 1)
[b] p<0.05 in comparison to the cage (group 2)
[c] p<0.05 in comparison to the PDLLA coated cage (group 3).

Table 5. After 12 Weeks, Flurochrome Analysis Was Performed

	Group 1 bone graft		Group 2 cage		Group 3 cage + PDLLA		Group 4 cage + PDLLA +IGF1+TGF-β1	
	Adjacent	Within	Adjacent	Within	Adjacent	Within	Adjacent	Within
3 weeks	0	0	1	1	0	0	2	2
6 weeks	1	0	1	5	3	6	4	4
9 weeks	1	0	1	6	5	6	6	6

Additionally, the growth factor group was compared with a group that underwent the "standard" cervical spine fusion technique using tricortical iliac crest bone graft.

As compared with the bone graft group (Group 1), the cage plus PDLLA plus IGF-I and TGF-β1 group (Group 4) demonstrated better maintenance of lordosis, a higher biomechanical stability, advanced interbody bone matrix formation in histomorphometric analysis, and accelerated interbody bone matrix formation on fluorochrome sequence labeling. Additionally, donor-side morbidity of the iliac crest graft was excluded. Yet, all the cage groups (Groups 2 to 4) investigated in this study outperformed the subjects who underwent tricortical iliac crest graft in terms of histomorphometric parameters and biomechanical stability in rotation. Therefore, these effects were predominantly associated with the use of the interbody fusion cage. However, these effects were supplemented and increased by the growth factors IGF-I and TGF-β1. As compared with the groups in which the uncoated (Group 2) and coated cages (Group 3) were used, the growth factor group (Group 4) showed a lower flexion–extension difference for the lordosis angle in functional radiographic evaluations, a higher bone mineral content and bony callus volume, a lower range of motion in rotation and bending, and a higher bone ratio of volume to total volume in the histomorphometric analysis.

These preliminary results are in concordance with those of previous animal studies using IGF-I and TGF- β1 in other anatomic locations. In these studies, IGF-I and TGF- β1 individually and in combination were shown to improve bone matrix formation and bone remodeling by direct and indirect mechanisms.[2,9,14,27,32,35,38,45]. Although no impressive differences in the fusion score could be determined between any of the groups (Table 6), in this 12-week follow-up study, the results demonstrate that combined IGF-I and TGF-β1 application was able to promote intervertebral bone matrix formation in the sheep cervical spine. These results are encouraging, but longer-term studies are essential to determine whether this growth factor combination also is able to promote solid bony fusion.

For systemically applied IGF-I or TGF-β1, side effects were described depending on concentration.27,44,47 Especially, IGF-I has been associated with a variety of systemic side effects and complications, including electrolyte shifts and hypoglycemia, resulting from serum concentrations of insulin or changes in serum concentrations of thyroid hormone, for example, parotid swelling and tachycardia.47 Additionally, systemically applied TGF- β1 was associated with increased serum concentrations of alkaline phosphatase.27 On the basis of these very preliminary results, the systemic side effects of IGF-I or TGF-β1 on blood count, electrolytes, glucose levels, alkaline phosphatase levels, and thyroid hormone concentrations could not be observed. Additionally, no changes in body weight, body temperature, or acute illness were detected. However, IGF-I or TGF-β1 clearly have not been proved safe for spinal application. Some studies, for example, demonstrated that TGF-ß1 has effects on neurologic tissue, especially on human glioblastoma cells, astrocytes, and Schwann cells.27,47 Therefore, further studies are necessary to address safety issues associated with isolated or combined spinal application of IGF-I and TGF- β1.

Table 6. Histologic Analysis of Interbody Fusion: After 12 Weeks, Bony Fusion of the 4 Groups Was Evaluated Using a 4-Point Scale According to Zdeblick[49]

Histologic Fusion Points [49]	Group 1 (n=8) Bone Graft	Group 1 (n=8) Cage	Group 1 (n=8) Cage + PDLLA	Group 1 (n=8) Cage + PDLLA + IGF-I + TGF-β1
0	0	0	0	0
1	2	1	1	0
2	5	6	6	5
3	1	0	1	2
4	0	0	0	1

If four points were awarded, a successful arthrodesis or fusion was considered to have occurred. Three points were graded as a developing fusion.

Conclusion

Findings show that PDLLA-coating of cervical spine interbody fusion cages as a delivery system for growth factors is effective. The slight but insignificant positive effect of the PDLLA carrier on interbody fusion might result from the degradation process of the biodegradable carrier.

As compared with the iliac crest graft, IGF-I and TGF- β1 application by a PDLLA-coated interbody cage significantly improved the results of interbody bone matrix formation while excluding the donor-side morbidity. Although the cage groups all outperformed the groups that underwent tricortical iliac crest bone graft in terms of histomorphometric parameters and biomechanical stability, these effects were supplemented and increased by the growth factors IGF-I and TGF- β1. However, in this short-term follow-up study, these growth factors were not able to increase the incidence of solid bony fusion. Therefore, longer-term studies are required to determine whether combined IGF-I and TGF-β1 application leads to a successful fusion.

Key Points

- The findings showed that PDLLA coating of cervical spine interbody fusion cages as a delivery system for growth factors was effective.
- Although IGF-I and TGF-ß1 application by a PDLLA-coated interbody cage was not able to achieve solid bony fusion during the 12-week follow-up period, these growth factors significantly increased the results of interbody bone matrix formation.
- Additional longer-term studies are required to determine whether combined IGF-I and TGF-ß1 application leads to a successful spinal fusion.

References

1. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R. Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and oestrogen deficiency. J Bone Miner Res 2000;15:683-69
2. Beck L, Amento E, Xu Y, Deguzman L, Lee W, Nguyen T, Gillet N. TGF-beta 1 induces bone closure of skull defects – Temporal dynamics of bone formation in defects exposed to rhTGF-beta 1. J Bone Miner Res 1993;8:753-761
3. Boden SD, Zdeblick TA, Sandhu HS, Heim SE. The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine 2000;25:376-381
4. Boden SD, Martin GJ Jr, Morone MA, Ugbo JL, Moskovitz PA. Posterolateral lumbar intertransverse process spine arthrodesis with recombinant human bone morphogenetic protein 2/hydroxyapatite-tricalcium phosphate after laminectomy in the nonhuman primate. Spine 1999;24:1179-1185
5. Brady JM, Cutright DE, Miller RA, Barristone GC. Resorption rate, route, route of elimination, and ultrastructure of the implant size of polylactic acid in the abdominal wall of the rat. J Biomed Mater Res 1973;7:155-166
6. Cutright DE, Hunsuck EE, Beasley JD. Fracture reduction using a biodegradable material, polylactic acid. J Oral Surg 1971;29:393-397
7. David SM, Gruber HE, Mayer RA Jr, Murakami T, Tabor OB, Howard BA, Wozney JM, Hanley EN Jr. Lumbar spinal fusion using recombinant human bone morphogenetic protein in the canine. A comparison of three dosages and two carriers. Spine 1999;24:1973-1979
8. Fischgrund JS, James SB, Chabot MC, Hankin R, Herkowitz HN, Wozney JM, Shirkhoda A. Augmentation of autograft using rhBMP-2 and different carrier media in the canine spinal fusion model. J Spinal Disord 1997;10:467-472
9. Fujimoto A, Tanizawa T, Nishida S, et al. Local effects of transforming growth factor-beta 1 on rat calvaria: Changes depending on the dose and the injection site. J Bone Miner Metab 1999; 17: 11–17.
10. Gombotz W, Pankey S, Bouchard L, et al. Controlled release of TGF-beta 1 from a biodegradable matrix for bone regeneration. J Biomater Sci Polym Ed 1993; 5: 49–63.
11. Gopferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996; 17: 103–14.
12. Hecht BP, Fischgrund JS, Herkowitz HN, et al. The use of recombinant human bone morphogenetic protein 2 (rhBMP-2) to promote spinal fusion in a nonhuman primate anterior interbody fusion model. Spine 1999; 24: 629–36.
13. Hermann R, Schmidmaier G, Markl B, et al. Antithrombogenic coating of stents using a biodegradable drug delivery technology. Thromb Haemost 1999; 82: 51–7.
14. Hock J, Centrella M, Canalis E. Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology 1988; 122: 254–60.
15. Hollinger JO, Battistone GC. Biodegradable bone repair materials: Synthetic polymers and ceramics. Clin Orthop 1986; 278: 290–305.
16. Hutmacher D, Hurzeler MB, Schliephacke H. A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. Int J Oral Maxillofac Implants 1996; 11: 667–78.
17. Isgaard J, Nilson A, Lindahl A, et al. Effects of local administration of GH and IGF-I on longitudinal bone growth in rats. Am J Physiol 1986; 250: 367–72.
18. Itoh H, Ebara S, Kamimura M, et al. Experimental spinal fusion with use of recombinant human bone morphogenetic protein 2. Spine 1999; 24: 1402–5.
19. Kandziora F, Kerschbaumer F, Starker M, et al. Biomechanical assessment of transoral plate fixation for atlantoaxial instability. Spine 2000; 25: 1555–61.
20. Kandziora F, Pflugmacher R, Scholz M, et al. The sheep cervical spine in comparison to the human spineA An anatomic, radiographic, bone mineral density and biomechanical study. Spine 2001; 26: 1028–37.
21. Kleemann R. Entwicklung eines Wirbelsäulenprüfstands zur Testung von Implantaten an der Halswirbelsäule: Semesterarbeit: Fakultät für Maschinenbau. Berlin: Technische Universität, 1999.
22. Kulkarni RK, Moore EG, Hegyeli AF, et al. Biodegradable poly(lactic acid) polymers. J Biomed Mater Res 1971; 5: 169–81.
23. Kulkarni RK, Pani KC, Neuman C, et al. Polylactic acid for surgical implants. Arch Surg 1966; 93: 839–43.
24. Kumta SM, Spinner R, Leung PC. Absorbable intramedullary implants for hand fractures: Animal experiment and clinical trial. J Bone Joint Surg [Br] 1992; 93: 839–43.
25. Laurencin C, Lane JM. Poly (lactide acid), poly (glycolid acid): Orthopedic surgery applications. In: Brighton C, Frielaender G, Lane MJ, eds. Bone Formation and Repair. Rosemont: American Academy of Orthopedic Surgeons, 1994: 325–39.
26. Lind M. Growth factor stimulation on bone healing: Effects on osteoblasts, osteotomies, and implants fixations. Acta Orthop Scand Suppl 1998; 283: 2–37.
27. Lind M, Schuhmacker B, Soballe K, et al. Transforming growth factor-beta enhances fracture healing in rabbit tibiae. Acta Orthop Scand 1993; 64: 553–6.
28. Majola A, Vainionpaa S, Vihtonen K, et al. Absorption, biocompatibility, and fixation properties of polylactic acid in bone tissue: An experimental study in rats. Clin Orthop, 1991:260–9.
29. Martin GJ Jr, Boden SD, Marone MA, et al. Posterolateral intertransverse process spinal arthrodesis with rhBMP-2 in a nonhuman primate: Important lessons learned regarding dose, carrier, and safety. J Spinal Disord 1999; 12: 179–86.
30. Meyer RA Jr, Gruber HE, Howard BA, et al. Safety of recombinant human bone morphogenetic protein-2 after

spinal laminectomy in the dog. Spine 1999; 24: 747–54.
31. Miller RA, Brady JM, Cutright DE. Degradation rates of oralresorbable implants (polylactates and polyglycolates): Rate modification with changes in PLA/PGA copolymer ratios. J Biomed Mater Res 1977; 11: 711–19.
32. Mohan S, Baylink D. Bone growth factors. Clin Orthop Rel Res 1991; 263: 30–48.
33. Nielson H, Isgaard J, Lindahl A, et al. Effects of unilateral arterial infusion of GH and IGF-I on tibial longitudinal bone growth in hypophysectomized rats. Calcif Tissue Int 1987; 40: 91–6.
34. Pfeilschifter J, Oechsner M, Naumann A, et al. Stimulation of bone matrix apposition *in vitro* by local growth factors: A comparison between insulin-like growth factor-I, platelet derived growth factor, and transforming growth factor-beta. Endocrinology 1990; 127: 69–75.
35. Roberts A, Sporn M, Bolander M. Transforming growth factor-beta and the initiation of chondrogenesis in the rat femur. J Cell Biol 1990; 110: 2195–207.
36. Sandhu HS, Kanim LE, Kabo JM, et al. Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion. Spine 1996; 21: 2115–22.
37. Sandhu HS, Kanim LE, Toth JM, et al. Experimental spinal fusion with recombinant human bone morphogenetic protein-2 without decortication of osseous elements. Spine 1997; 22: 1171–80.
38. Schmidmaier G, Wildemann B, Bail HJ, et al. Local application of growth factors IGF-I and TGF-ß1 from a biodegradable poly-(D,L-lactide) coating of osteosynthetic implants accelerate fracture healing. Chirurg 2000; 71: 1016–22.
39. Schmidmaier G, Wildemann B, Stemberger A, et al. Biodegradable poly-(D,L-lactide) coating of steel and titanium implants for continuous release of growth factors (IGF-I and TGFß1). Bone (accepted).
40. Sorensen TS, Sorensen AI, Merser S. Rapid release of gentamicin from collagen sponge: *In vitro* comparison with plastic beads. Acta Orthop Scand 1990; 61: 353–6.
41. Steinbrech DS, Mehrara BJ, Rowe NM, et al. Gene expression of TGF-beta, TGF-beta receptor, and extracellular matrix proteins during membranous bone healing in rats. Plast Reconstr Surg 2000; 105: 2028–38.
42. Takaoka K, Koezuka M, Nakahara H. Telepeptide-depleted bovine skin collagen as a carrier for bone morphogenetic protein. J Orthop Res 1991; 9: 902–7.
43. Tamada JA, Langer R. Erosion kinetics of hydrolytically degradable polymers. Proc Natl Acad Sci U S A 1993; 90: 552–6.
44. Terrell TG, Working PK, Chow CP, et al. Pathology of recombinant human transforming growth factor-beta 1 in rats and rabbits. Int Rev Exp Pathol 1993; 34B: 43–67.
45. Thaller S, Hoyt J, Tesluck H, et al. The effect of insulin growth factor-I on calvarial sutures in Sprague-Dawley rat. J Craniofac Surg 1993; 4: 35–9.
46. Trippel S, Coutts R, Einhorn T, et al. Growth factors as therapeutic agents. J Bone Joint Surg [Am] 1996; 78: 1272–86.
47. Wilton P. Treatment with recombinant human insulin-like growth factor-I of children with growth hormone receptor deficiency (Laron syndrome). Kabi Pharmacia Study Group on insulin-like growth factor-I treatment in growth hormone insensitivity syndromes. Acta Paediatr Suppl 1992; 383: 137–42.
48. Yamada Y, Harada A, Hosoi T, et al. Association of transforming growth factor beta-1 genotype with therapeutic response to active vitamin D for postmenopausal osteoporosis. J Bone Miner Res 2000; 15: 415–20.
49. Zdeblick TS, Ganayem AJ, Rapoff AJ, et al. Cervical interbody fusion cages: An animal model with and without bone morphogenetic protein. Spine 1998; 23: 758–65.
50. Zegzula HD, Buck DC, Brekke J, et al. Bone formation with use of rhBMP-2 (recombinant human bone morphogenetic protein-2). J Bone Joint Surg [Am] 1997; 79: 1778–90.

Eur Spine J (2002) 11 : 482–493
DOI 10.1007/s00586-001-0384-4

ORIGINAL ARTICLE

F. Kandziora
R. Pflugmacher
M. Scholz
C. Knispel
T. Hiller
G. Schollmeier
H. Bail
G. Schmidmaier
G. Duda
M. Raschke
N. P. Haas

Comparison of BMP-2 and combined IGF-I/TGF-ß1 application in a sheep cervical spine fusion model

Received: 16 July 2001
Revised: 8 October 2001
Accepted: 21 December 2001
Published online: 14 March 2002

This study was supported by MBI – Max Biedermann Institut der Steinbeis Stiftung (RS/KF-82042).

F. Kandziora (✉) · R. Pflugmacher
M. Scholz · C. Knispel · T. Hiller
G. Schollmeier · H. Bail · G. Schmidmaier
G. Duda · M. Raschke · N.P. Haas
Department of Trauma
and Reconstructive Surgery,
Charité University Hospital
of the Humboldt University Berlin,
Campus Virchow-Klinikum,
Augustenburgerplatz 1,
13353 Berlin, Germany
e-mail: frank.kandziora@charite.de,
Tel.: +49-30-450552012,
Fax: +49-30-450552901

Abstract Growth factors have proven to promote spine fusion. However, no comparative evaluation of growth factors in spinal fusion has yet been performed. The purpose of this study was to compare the efficacy and safety of combined IGF-I and TGF-ß1 application with BMP-2 application and autologous cancellous bone graft at an early time point in a sheep cervical spine fusion model. Thirty-two sheep underwent C3/4 discectomy and fusion. They were divided into four groups, according to their treatment: group 1, titanium cage (*n*=8); group 2, titanium cage filled with autologous cancellous iliac crest bone grafts (*n*=8); group 3, titanium cage coated with a poly-(D,L-lactide) (PDLLA) carrier including BMP-2 (5% w/w) (*n*=8); group 4, titanium cage coated with a PDLLA carrier including IGF-I (5% w/w) and TGF-ß1 (1% w/w) (*n*=8). Blood samples, body weight and temperature were analysed. Radiographic scans were performed pre- and postoperatively and after 1, 2, 4, 8 and 12 weeks. At the same time points, disc space height and intervertebral angle were measured. After 12 weeks, the animals were killed and fusion sites were evaluated using functional radiographic views in flexion and extension. Quantitative computed tomographic scans were performed to assess bone mineral density, bone mineral content and bony callus volume. Biomechanical testing was carried out and the values for range of motion, and neutral and elastic zone were determined. Histomorphological and histomorphometrical analysis were performed and polychrome sequential labelling was used to determine the time frame of new bone formation. The results showed that, in comparison to the group treated with the cage alone (group 1), the cage plus BMP-2 group (group 3) and the cage plus IGF-I and TGF-ß1 group (group 4) demonstrated a significantly higher fusion rate in radiographic findings, a higher biomechanical stability, a more advanced interbody fusion in histomorphometrical analysis, and an accelerated interbody fusion on fluorochrome sequence labelling. In comparison to the bone graft group (group 2), the BMP-2 (group 3) and IGF-I/TGF-ß1 group (group 4) showed significantly less residual motion on functional radiographic evaluation, higher bone mineral density of the callus and higher biomechanical stability in extension, rotation and bending. The BMP-2 group showed significantly less residual motion on functional radiographic evaluation and higher intervertebral bone matrix formation on fluorochrome sequence labelling at 9 weeks in comparison to the IGF-I/TGF-ß1 group. In contrast, the IGF-I/TGF-ß1 group showed a significantly higher bone mineral density of the callus than the BMP-2

group. In comparison to the autologous cancellous bone graft group, both growth factors (BMP-2 and combined IGF-I and TGF-ß1) significantly improved the biomechanical results of interbody fusion. No systemic side effects were observed for either growth factor. On the basis of these preliminary results, it would appear that combined IGF-I/TGF-ß1 application yields equivalent results to BMP-2 application at an early time point in anterior sheep cervical spine fusion.

Keywords Cervical spine · Sheep · Animal model · Interbody fusion · BMP-2 · IGF-I · TGF-ß1 · Growth factor

Introduction

More than 30 years ago, Urist [42] determined the osteoinductive capacity of demineralized bone matrix. Advances in protein isolation and molecular cloning technology subsequently yielded several soluble, low-molecular-weight growth factors, such as transforming growth factors (TGFs), bone morphogenetic proteins (BMPs), platelet-derived growth factors (PDGFs), insuline-like growth factors (IGFs), fibroplast growth factors (FGFs) and epidermal growth factors (EGFs) [41]. Meanwhile, many of these growth factors have been produced as recombinant molecules in virtually unlimited quantities using genetically modified cell lines. However, only some of them have demonstrated a significant osteoinductive capacity, and only two have been precisely evaluated in experimental spine fusion. Presently, only BMP-2 [4, 5, 6, 7, 8, 11, 16, 26, 27, 33, 34, 45, 46] and OP-1 (BMP-7) [10, 23] have proven to accelerate spinal fusion and to overcome the disadvantages of an autologous bone graft. Therefore, the optimum growth factor or growth factor combination to promote spinal fusion is still a matter of discussion.

Recently, in vitro and in vivo studies have demonstrated an osteoinductive effect of isolated IGF-I and TGF-ß1 or combined IGF-I/TGF-ß1 application [24, 25, 29, 41]. IGF-I stimulates the replication of osteoblasts and the synthesis of bone matrix [13]. TGF-ß1 regulates different cell types that are directly involved in bone remodeling and bone matrix formation, such as mesenchymal cells, chondrocytes, osteoblasts, and osteoclasts [31, 32]. In vivo studies have shown that decreased levels of IGF-I and TGF-ß1 are associated with bone loss and osteoporosis [1, 7, 44], whereas the local application of isolated IGF-I or TGF-ß1 can positively influence fracture healing [15, 30, 38]. Additionally, in vitro and in vivo studies have demonstrated a significant osteoinductive effect of combined IGF-I and TGF-ß1 application [20, 36]. Schmidmaier [36] demonstrated that the combined application of IGF-I and TGF-ß1 had a significantly higher stimulating effect on bone matrix formation in rat tibia fractures than a single application of IGF-I or TGF-ß1. Kandziora [20] was able to show an increased intervertebral bone matrix formation comparing combined IGF-I and TGF-ß1 application with an autologous tricortical iliac crest bone graft in a sheep cervical spine fusion model.

Presently, no comparative evaluations of different growth factors in experimental spine fusion are available. The purpose of this study was to compare the efficacy and safety of combined IGF-I and TGF-ß1 application with BMP-2 application and autologous cancellous bone grafts at an early time point in an in vivo sheep cervical spine interbody fusion model.

Materials and methods

Study design

Thirty-two adult female merino sheep (2 years old) underwent C3/4 discectomy and fusion. No sheep was lost to follow-up. The sheep were randomly assigned to the following groups:

- Group 1: titanium cage (n=8)
- Group 2: titanium cage filled with autologous cancellous iliac crest bone grafts (n=8)
- Group 3: titanium cage coated with a biodegradable poly-(D,L-lactide) (PDLLA) carrier including BMP-2 (5% w/w) (n=8)
- Group 4: titanium cage coated with a biodegradable PDLLA carrier including IGF-I (5% w/w) and TGF-ß1 (1% w/w) (n=8)

After 12 weeks all the sheep were sacrificed and radiographic, biomechanical and histological evaluations were performed. All animal experimental work was approved by local authorities.

Coating of the cages

PDLLA (Boehringer Ingelheim, Germany) was chosen as the drug carrier system. The properties of the PDLLA coating and the coating technique have been described previously [12, 35]. In group 3, recombinant human BMP-2 (rhBMP-2, R&D Systems, Abingdon, UK) (5% w/w) was incorporated in the PDLLA coating. In group 4, recombinant human IGF-I (R&D Systems) (5% w/w) and recombinant human TGF-ß1 (R&D Systems) (1% w/w) were incorporated in the PDLLA coating. The average PDLLA coating mass of the cages was 3.02±0.12 mg. Therefore, approximately 150 µg (5% w/w) BMP-2 and 150 µg (5% w/w) IGF-I plus 30 µg (1% w/w) TGF-ß1 were incorporated in the coating of each cage in the respective groups.

Surgical technique and postoperative care

The animals underwent the surgical procedure under general endotracheal anaesthesia. The anterior part of the neck and the left iliac crest (group 2) was prepped in a sterile fashion and a left anterolateral approach to the cervical spine was carried out through a longitudinal skin incision. The longus colli muscle was incised in the midline, and the intervertebral disc C3/4 was exposed. After distraction of the motion segment with a Caspar distractor, anterior discectomy C3/4 was performed. The endplates were uniformly shaved with a 2-mm high-speed diamond drill down to bleeding

bone. For interbody stabilization, meshed titanium cages (Motech GmbH, Schwenningen, Germany, height 8 mm, diameter 14 mm) were used. In group 2, the cages were filled with autologous cancellous bone grafts taken from the left iliac crest. Prior to insertion, the volume of the bone grafts was determined using the water displacement technique (Archimedes principle). The average volume of the bone grafts was 1.42±0.1 cm^3. In the growth factor groups and the cage alone group (groups 1, 3 and 4) the cages were not filled with bone graft. Finally, all cages were inserted uniformly into the intervertebral space. The wound was irrigated with saline, and the longus colli muscle, the subcutaneous tissue and the skin were reapproximated with sutures and a soft bandage was applied to the neck.

After surgery, the animals were maintained under observation until fully recovered from general anaesthesia. They received two doses of 0.5 g metamizol-natrium (Novaminsulfon, Lichtenstein) per day for 5 days intramuscularly. Clinical examination was performed daily for the first 10 days, then weekly. The sheep were allowed ad libitum activity for the remainder of the experiment. Fluorochrome sequential labels were administered at 3, 6 and 9 weeks postoperatively, consisting of oxytetracycline (25 mg/kg IV) at 3 weeks, calcein green (15 mg/kg IV) at 6 weeks, and xylenol orange (90 mg/kg IV) at 9 weeks. Twelve weeks after surgery, the animals were killed after induction of anaesthesia by an intravenous injection of potassium chloride. The complete cervical spine including parts of the occiput and T1 was then excised and cleaned from the surrounding tissue.

Blood and serum analysis

Blood and serum samples were taken from the saphenous vein of the hind leg of the sheep pre- and postoperatively, and after 1, 2, 4, 8 and 12 weeks. The blood samples were analysed for routine laboratory parameters (blood count, electrolytes, alkaline phosphatase, thyroid values and glucose).

Body weight and body temperature

Preoperatively and after 1, 2, 4, 8 and 12 weeks, rectal body temperature and body weight were determined.

Radiographic analysis

Radiographic evaluations have been described in detail earlier [18]. Lateral and posteroanterior digital radiographic scans (X-ray unit: Mobilett Plus, Siemens AG, Germany; X-ray films: Fuji CR 24×30, Fuji, Germany) were performed pre- and postoperatively and after 1, 2, 4, 8 and 12 weeks. At the same time points, anterior, middle and posterior intervertebral disc space heights (DSH) and intervertebral angle (IVA) of the motion segment C3/4 were measured on lateral radiographic scans. Average intervertebral DSH was calculated from anterior, middle and posterior DSH measurements (anterior+middle+posterior DSH/3). After 12 weeks, bony fusion was categorised on lateral radiographs using the following parameters [20]: (A) no bony fusion, (B) maximum intervertebral gap in the cranio-caudal direction of more than 5 mm, (C) maximum intervertebral gap in the cranio-caudal direction of less than 5 mm, (D) complete bony fusion. The maximum intervertebral gap in the cranio-caudal direction was measured directly on lateral radiographs using a ruler. All radiographic measurements were evaluated by three independent observers.

Functional radiographic analysis

Functional radiographic evaluation of the sheep cervical spine has been described in detail earlier [18]. After sacrifice, fusion sites were evaluated using lateral digital functional radiographic scans in flexion and extension (X-ray unit: Mobilett Plus, Siemens AG, Germany; X-ray films: Fuji CR 24×30, Fuji, Germany). For this purpose, T1 was rigidly fixed with a Steinmann pin, while a 60-N load was applied through C1 using a dynamometer (Newtonmeter, Inha GmbH, Berlin, Germany). Flexion/extension differences in intervertebral angle (IVA) and lordosis angle (LA) were calculated. All functional radiographic measurements were evaluated by three independent observers.

Quantitative computed tomographic analysis

Quantitative computed tomographic scans (QCT) were performed using a Siemens Somatom plus 4 scanner (Siemens Inc., Erlangen, Germany). Axial cuts with 1-mm slice thickness were made parallel to the intervertebral disc space. Bone mineral density (BMD) and bone volume measurements of the callus have been described in detail earlier [18]. BMD measurements were calibrated with a six-point bone mineral density phantom, and were performed using the specific software of the scanner (Sienet Magic View VA 30A, Siemens, Inc.). Bony callus volume (BCV) was measured using an image analysing system (Zeiss KS 400, Zeiss GmbH, Germany). Bone mineral content (BMC) was calculated from BMD and BCV measurements (BMC=BCV×BMD). After 12 weeks, bony fusion was categorised on sagittal and coronal two-dimensional (2D) CT reconstructions using the A–D parameters described earlier [20]. The maximum intervertebral gap in the craniocaudal direction was measured directly on midsagittal 2D CT reconstructions using the scanner software described above. All radiographic CT measurements were evaluated by three independent observers.

Biomechanical analysis

After euthanasia, biomechanical testing was performed by a nondestructive flexibility method using a nonconstrained testing apparatus described in detail earlier [17, 18]. Pure bending moments of 6 Nm were applied to the motion segments C3/4 using a system of cables and pulleys to induce flexion, extension, left and right lateral bending and left and right axial rotation. Tension was applied to the cables with a uniaxial testing machine (1456, Zwick GmbH, Ulm, Germany). Three-dimensional displacement of each motion segment was measured using an optical measurement system (Qualysis Inc., Sävebalden, Sweden). Triangular markers with three diodes (Qualysis Inc.) were attached to the bodies of C3 and C4. Marker positions were detected with two cameras and recorded with a computerized motion analysis system (PC-Reflex, Qualysis Inc). Angular displacement of the upper vertebra (C3) in relation to the lower vertebra (C4) was calculated from marker positions using custom-made computer software. The measurement error associated with this method was ±0.1° [21]. Range of motion (ROM), and neutral (NZ) and elastic (EZ) zones were determined.

Histomorphological, histomorphometrical and fluorochrome analysis

All C3/4 motion segments were harvested at 12 weeks for bone histology. The motion segments had been fixed for 7 days in 10% normal buffered formaldehyde followed by dehydration in ascending concentrations of ethanol, and embedded undecalcified in methylmethacrylate (Technovit 9100, Heraeus Kulzer GmbH, Germany).

For histomorphological and histomorphometrical analysis, longitudinal sections in the sagittal plane were cut at 6 µm with a Leica SM 2500 S microtome and a 40° stainless steel knife. Afterwards, the residual parts of the cages were removed and the following stains were used: (1) Safranin-O/Lightgreen, (2) Safranin-O/v. Kossa, (3) Astrablue and (4) Masson-Goldner.

Masson-Goldner stainings were used for histomorphological analysis, which included evaluation of bony fusion using the A–D parameters described earlier [20]. The maximum intervertebral gap in the cranio-caudal direction was measured directly on mid-sagittal sections using an image analysing system (Zeiss KS 400, Zeiss GmbH, Germany). Histomorphometrical parameters were measured on the residual stainings using a Leica DM-RB microscope, and the image analysing system (Zeiss KS 400). Parameters were measured at a magnification of ×1.6.

The sagittal diameter distance (S) of C3 and the average preoperative DSH were determined to define the size of the region of interest (ROI) for histomorphometrical evaluation [20]. The entire intervertebral fusion area was included in this ROI. The following structural indices were calculated in the ROI: bone volume/total volume (BV/TV), cartilage volume/total volume (CV/TV), mineralised cartilage volume/cartilage volume (mCV/CV).

For fluorochrome analysis, longitudinal sections in the parasagittal plane were cut at 400 µm with a precise macro grinding machine (Fa. Exact, Norderstedt, Germany). These slices were then ground to a thickness of 80 µm using a precise micro grinding machine (Fa. Exact). Fluorochrome markers were analysed under appropriate lighting conditions using a Leica DM-RB microscope and an image analysing system (Zeiss KS 400). Parameters were measured at a magnification of ×1.6.

Fluorochrome analysis of intervertebral fusion areas has been described in detail earlier [45]. The first appearance of the marker served to time formation of new bone matrix. The presence or absence of each marker around or within the cage was used to determine the relative time frame of new bone formation.

Statistical analysis

Comparison of data was performed using one way ANOVA for independent samples followed by TUKEY post-hoc analysis for multiple comparison procedures with Bonferroni correction for multiple measurements. Intraobserver variability for radiographic measurements, functional radiographic evaluation and CT measurements was determined using kappa statistics. The A–D score [20] was used to categorise semiquantitative bony fusion on plain radiographs, CT scans and histological stainings; however, no statistical evaluation of this score was performed. Statistically significant differences were defined at a 95% confidence level. The values are given as mean±SD. SPSS (release 7.0, SPSS Inc., Chicago, Illinois) software supported statistical evaluation.

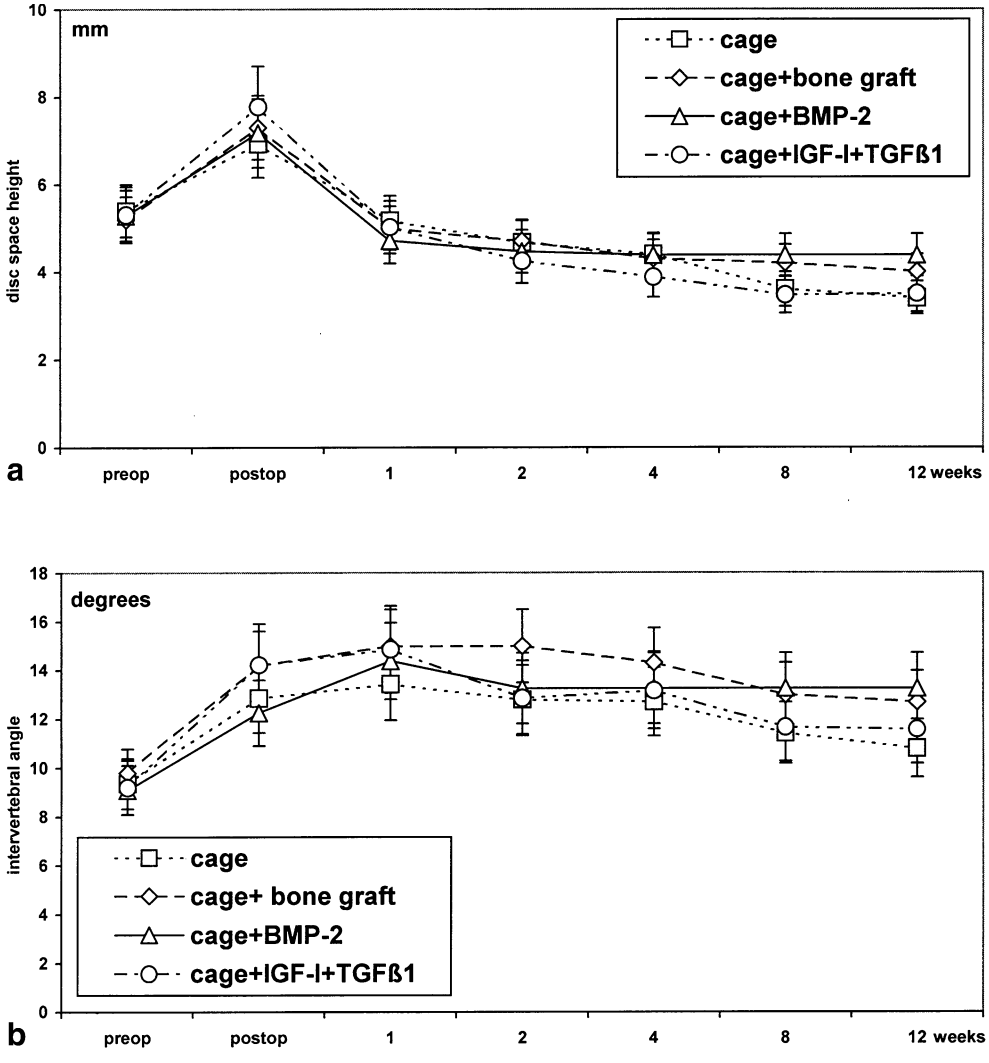


Fig. 1a, b Radiographic analysis. **a** Average disc space height of the different groups throughout the observation period. **b** Average intervertebral angle of the different groups throughout the observation period

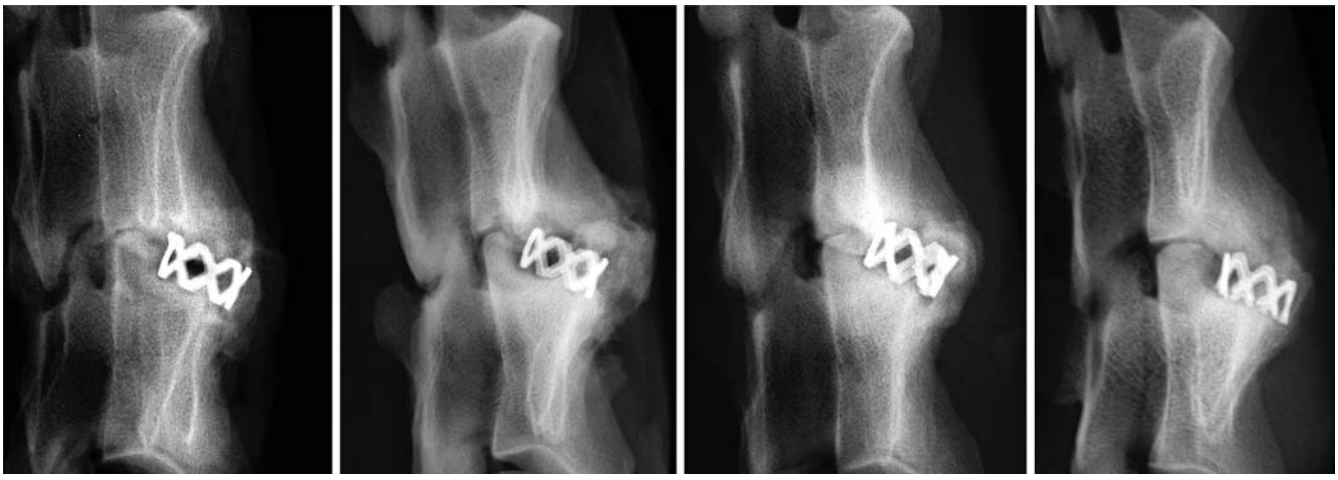


Fig. 2 Radiographic analysis. After 12 weeks, bony fusion was evaluated on lateral radiographic scans (animal 8 of each group)

Results

Blood and serum analysis results

Full blood count did not show any significant differences either between the groups or at different time points throughout the course of the experiment. Even in the initial period, 1 week postoperatively, no significant changes were found for average haemoglobin, erythrocyte and leukocyte levels compared to the preoperative baseline levels.

Levels of electrolytes (Na, K, Cl, Ca) did not show significant changes during the experimental period. Furthermore, no differences in thyroid hormones, alkaline phosphatase or glucose levels were found throughout the observation period or between the groups.

Body weight and body temperature

No significant differences were found between all groups in mean body temperature and body weight throughout the experimental period. Postoperatively, a slight and constant increase in body weight for all sheep of all groups was determined.

Radiographic results

Intraobserver agreement for radiographic measurements was good, showing kappa values ranging between 0.74 and 0.93.

Preoperative baseline values of all radiographic parameters did not show any differences between the groups. No significant differences were found for average disc space height and intervertebral angle between all groups throughout the observation period (Fig. 1). However, at 12 weeks there was a trend for a smaller intervertebral angle and disc space height in the cage alone group (group 1) compared to the other groups.

After 12 weeks, bony fusion was evaluated on radiographic scans (Fig. 2). In the bone graft group (group 2), the BMP-2 group (group 3) and the IGF-I/TGF-ß1 group (group 4), a more advanced interbody fusion was found in comparison to the cage alone group (Table 1). No noticeable difference in fusion rate was found between the bone graft group (group 2) and the BMP-2 group (group 3) or the IGF-I/TGF-ß1 group (group 4).

Functional radiographic results

Intraobserver agreement for functional radiographic measurements was excellent, showing kappa values ranging between 0.86 and 0.93.

After 12 weeks there were no significant differences between flexion/extension values of intervertebral and lordosis angle (Table 2) between the cage alone group (group 1) and the cage plus bone graft group (group 2).

Table 1 Radiographic analysis. After 12 weeks, bony fusion of the four groups was determined on radiographic scans using the following parameters: (A) no bony fusion, (B) maximum intervertebral gap in cranio-caudal direction of more than 5 mm, (C) maximum intervertebral gap in cranio-caudal direction of less than 5 mm, (D) complete bony fusion

Bony fusion parameter (score)	Group 1 (n=8) Cage	Group 2 (n=8) Cage+bone graft	Group 3 (n=8) Cage+ BMP-2	Group 4 (n=8) Cage+IGF-I+ TGF-ß1
A	0	0	0	0
B	6	4	2	2
C	2	3	4	5
D	0	1	2	1

Table 2 After 12 weeks, functional radiographic evaluation in flexion/extension of the four groups was determined. Flexion/extension differences of intervertebral angle (IVA) and lordosis angle (LA) were calculated: values are presented as mean ±SD, with range in parentheses

Flexion/ extension difference in degrees	Group 1 (n=8) Cage	Group 2 (n=8) Cage+bone graft	Group 3 (n=8) Cage+ BMP-2	Group 4 (n=8) Cage+IGF-I+ TGF-ß1
IVA	8.6±2.6 (6.0–10.0)	7.7±3.0 (4.5–11.0)	2.7±2.1[a,b,c] (0–7.0)	5.9±1.9[a] (3.5–7.5)
LA	8.6±3.1 (6.0–12.0)	7.9±3.4 (4.5–11.5)	3.9±2.3[a,b,c] (0–9.0)	6.3±1.6[a] (5.0–8.0)

[a] P<0.05 in comparison to the cage alone group (group 1)
[b] P<0.05 in comparison to the cage plus bone graft group (group 2)
[c] P<0.05 in comparison to the cage plus IGF-I/TGF-ß1 group (group 3)

Flexion/extension differences in the IGF-I/TGF-ß1 group (group 4) were significantly lower than in group 1 (P<0.05). Functional radiographic assessment revealed significantly lower residual flexion/extension movement in the cages with BMP-2 group (group 3) than in any other group (P<0.05).

Quantitative computed tomographic results

Intraobserver agreement for CT measurements was excellent, showing kappa values ranging between 0.84 and 0.98.

After 12 weeks, bone mineral content (BMC) and bony callus volume (BCV) were significantly lower in the cage alone group (group 1) than in any other group (P<0.05) (Fig. 3). There were no significant differences in BMC and BCV between the cage plus bone graft group (group 2), the cage plus BMP-2 group (group 3) and the cage plus TGF-I/IGF-ß1 group (group 4). Bone mineral density of the callus (BMD) in the cage plus IGF-I/TGF-ß1 group (group 4) was significantly higher than in any other group (P<0.05). The cages with BMP-2 group (group 3) showed significantly higher values for BMD (P<0.05) than groups 1 and 2 (Table 3). There was no significant difference for BMD of the callus between the cage alone group (group 1) and the cages filled with cancellous bone grafts (group 2).

Fusion was evaluated on sagittal 2D CT reconstructions (Table 4). In the bone graft group (group 2), the BMP-2 group (group 3) and the IGF-I/TGF-ß1 group (group 4), a more advanced interbody fusion was found in comparison to the cage alone group (Table 1). No significant difference in fusion score was found between the bone graft group (group 2) and the BMP-2 group (group 3) or the IGF-I/TGF-ß1 group (group 4).

Biomechanical results

Biomechanical results for range of motion (ROM), neutral zone (NZ) and elastic zone (EZ) are depicted in Table 5.

Lowest ROM, NZ and EZ values were consistently found for the growth factor groups (groups 3 and 4). ROM in all directions, NZ and EZ in rotation and NZ in lateral bending were significantly (P<0.05) lower in the growth factor groups (groups 3 and 4) than in the cage alone group (group 1) and the cage plus bone graft group (group 2). No significant difference for ROM, NZ and EZ in any direction was found between the cage alone group (group 1) and the cage plus bone graft group (group 2). Additionally, no significant difference was found between the cage plus BMP-2 group (group 3) and the cage plus IGF-I/TGF-ß1 group (group 4).

Histomorphological results

Histomorphological analysis supported the findings of radiographic and biomechanical examinations (Fig. 4).

In the cage alone group (group 1), mainly fibroblasts and occasionally cartilage cells were observed between the endplates. Cages were surrounded by a distinct small line of fibroblasts. Group 2, stabilised with cages plus cancellous bone grafts, showed some bony islands between the endplates with cartilage and fibrous tissue com-

Fig. 3 Computed tomographic (CT) analysis. After 12 weeks, interbody fusion was evaluated using quantitative computed tomography (QCT). Depicted are axial CT scans performed parallel to the intervertebral space (animal 6 of each group)

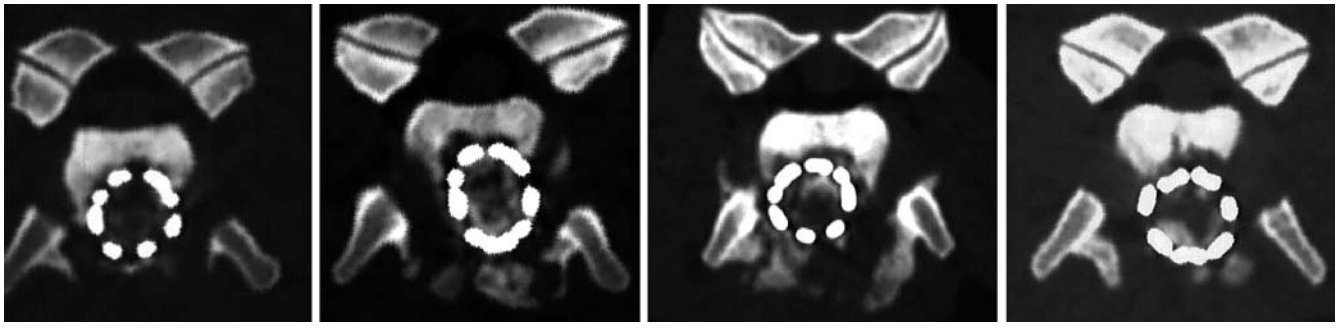


Table 3 After 12 weeks, quantitative computed tomographic analysis (QCT) was performed to measure bone mineral density (BMD), bone mineral content (BMC) and bony callus volume (BCV): values are presented as mean ±SD, with range in parentheses

QCT	Group 1 (*n*=8) Cage	Group 2 (*n*=8) Cage+bone graft[a]	Group 3 (*n*=8) Cage+ BMP-2	Group 4 (*n*=8) Cage+IGF-I+ TGF-ß1
BMD (g/cm^3)	0.58±0.04 (0.65–0.56)	0.55±0.05 (0.52–0.59)	0.62±0.04[b,c] (0.65–0.58)	0.65±0.04[b,c,d] (0.68–0.59)
BMC (g)	1.9±0.7 (1.2–2.8)	3.2±0.3[b] (2.8–3.7)	3.1±1.1[b] (2.0–5.5)	3.4±1.6[b] (2.4–5.5)
BCV (cm^3)	3.3±1.2 (2.0–4.7)	5.4±1.4[b] (3.8–6.3)	5.4±1.9[b] (2.1–7.4)	5.2±1.2[b] (4.1–6.8)

[a] Initial callus volume of the bone graft was 1.42 cm^3
[b] $P<0.05$ in comparison to the cage alone group (group 1)
[c] $P<0.05$ in comparison to the cage plus bone graft group (group 2)
[d] $P<0.05$ in comparison to the cage plus BMP-2 group (group 3)

Table 4 CT evaluation. After 12 weeks, bony fusion of the four groups was determined on sagittal two-dimensional CT reconstruction using the following parameters: (A) no bony fusion, (B) maximum intervertebral gap in cranio-caudal direction of more than 5 mm, (C) maximum intervertebral gap in cranio-caudal direction of less than 5 mm, (D) complete bony fusion

Bony fusion parameter (score)	Group 1 (*n*=8) Cage	Group 2 (*n*=8) Cylinder cage+ bone graft	Group 3 (*n*=8) Cage+ BMP-2	Group 4 (*n*=8) Cage+PDLLA+ IGF-I+TGF-ß1
A	0	0	0	0
B	6	4	2	2
C	2	3	5	5
D	0	1	1	1

ponents. The tissue surrounding the cages appeared similar to that in group 1. Inside the cage of group 2 sheep, osteoclastic activity was noted as an indication of graft resorption. Groups 3 and 4, stabilised with cages coated with BMP-2 and IGF-I/TGF-ß1, respectively, showed extensive callus formation and bony islands between the endplates, with cartilage and small fibrous tissue components. Most of the callus was seen ventrally. These findings were accompanied by capillary ingrowth and resorptive lacunae, without major differences between the two growth factor groups. No ossifications of the spinal ligaments were observed in any group.

Bony fusion was evaluated histomorphologically (Table 6). No major differences in the fusion score were found between the bone graft group (group 2) and the BMP-2 group (group 3) or the IGF-I/TGF-ß1 group (group 4). However, in comparison to the cage alone group (group 1), all these groups showed more advanced interbody bone matrix formation.

Table 5 Biomechanical analysis. After 12 weeks, biomechanical analysis was performed to measure range of motion (ROM), neutral (NZ) and elastic zone (EZ) for the different test modes: values (in degrees) are presented as mean ±SD

Test modes	Group 1 (*n*=8) Cage	Group 2 (*n*=8) Cage+bone graft	Group 3 (*n*=8) Cage+ BMP-2	Group 4 (*n*=8) Cage+IGF-I+ TGF-ß1
Flexion				
ROM	3.9±0.8	3.9±1.3	2.9±1.2	2.9±1.2
NZ	1.3±0.6	1.3±0.9	0.9±1.1	0.7±0.7
EZ	2.6±0.8	2.6±0.8	2.0±0.7	2.3±0.8
Extension				
ROM	3.9±1.3	3.8±1.2	2.7±0.7[a]	2.2±0.7[a,b]
NZ	1.0±0.6	1.4±0.9	0.6±0.5	0.6±0.5
EZ	2.9±0.9	2.4±1.0	2.1±0.3[a]	1.6±0.3[a,b]
Right rotation				
ROM	2.3±1.0	2.0±1.0	1.0±0.3[a,b]	1.4±0.3[a]
NZ	0.5±0.1	0.4±0.2	0.2±0.1[a,b]	0.2±0.1[a,b]
EZ	2.0±0.9	1.6±0.9	0.8±0.3[a,b]	1.2±0.3[a]
Left rotation				
ROM	2.3±0.8	2.1±1.0	1.1±0.4[a,b]	1.5±0.4[a]
NZ	0.4±0.1	0.5±0.3	0.2±0.1[a,b]	0.2±0.1[a,b]
EZ	1.9±0.7	1.6±0.9	0.9±0.4[a,b]	1.3±0.3[a]
Right bending				
ROM	3.8±1.0	4.2±2.2	2.1±0.7[a,b]	2.5±0.8[a,b]
NZ	1.0±0.4	1.2±1.2	0.6±0.2[a,b]	0.6±0.3[a,b]
EZ	2.8±0.7	3.0±1.9	1.5±0.7[a,b]	1.9±0.7[a,b]
Left bending				
ROM	3.9±1.2	4.1±2.1	2.1±0.9[a,b]	2.5±0.8[a,b]
NZ	1.0±0.4	1.3±1.2	0.5±0.2[a,b]	0.5±0.4[a,b]
EZ	2.9±0.8	2.8±1.9	2.0±0.7[a,b]	2.0±0.5[a,b]

[a] $P<0.05$ in comparison to the cage alone group (group 1)
[b] $P<0.05$ in comparison to the cage plus bone graft group (group 2)

Histomorphometrical results

The results of histomorphometrical analysis are presented in Table 7. Histomorphometrical analysis showed no significant differences in sagittal diameter index (baseline) between the groups. Compared to the cage alone group (group 1), histomorphometrical parameters revealed significantly more advanced bone matrix formation (bone volume/total volume ratio) in groups 2, 3 and 4 ($P<0.05$). No differences were found in the bone volume/total volume ratio between the bone graft group (group 2) and the BMP-2 group (group 3) or the IGF-I/TGF-ß1 group (group 4). There were no differences in the other histomorphometrical parameters for any of the groups.

Fluorochrome analysis results

The results of fluorochrome analysis are depicted in Table 8. The BMP-2 and IGF-I/TGF-ß1 coated cages exhibited

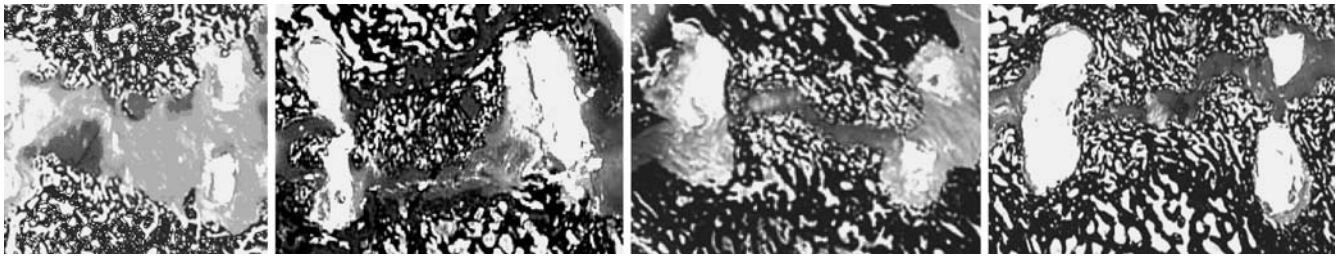


Fig. 4 Histomorphological analysis of the intervertebral fusion area. After 12 weeks, interbody fusion was evaluated histomorphologically and histomorphometrically on midsagittal slides. Depicted is the intervertebral bone matrix formation within the cage (animal 6 of each group; Safranin O/v. Kossa staining, magnification ×2.4)

Table 6 Histomorphological analysis. After 12 weeks, bony fusion of the four groups was evaluated histomorphologically using the following parameters: (A) no bony fusion, (B) maximum intervertebral gap in cranio-caudal direction of more than 5 mm, (C) maximum intervertebral gap in cranio-caudal direction of less than 5 mm, (D) complete bony fusion

Bony fusion parameter (score)	Group 1 (*n*=8) Cage	Group 2 (*n*=8) Cage+bone graft	Group 3 (*n*=8) Cage+ BMP-2	Group 4 (*n*=8) Cage+IGF-I+ TGF-ß1
A	0	0	0	0
B	6	4	3	3
C	2	3	4	4
D	0	1	1	1

Table 7 After 12 weeks, histomorphometrical analysis was performed and the following structural indices were calculated in the region of interest (ROI): sagittal diameter distance (SDD, baseline), bone volume/total volume (BV/TV), cartilage volume/total volume (CV/TV), mineralised cartilage volume/cartilage volume (mCV/CV): values are presented as mean ±SD, with range in parentheses

	Group 1 (*n*=8) Cage	Group 2 (*n*=8) Cage+bone graft[a]	Group 3 (*n*=8) Cage+ BMP-2	Group 4 (*n*=8) Cage+IGF-I+ TGF-ß1
SDD (mm)	25.6±1.2 (24.6–27.0)	25.5±1.1 (24.6–27.8)	25.8±1.4 (24.7–27.9)	26.6±1.1 (25.3–28.3)
BV/TV (%)	38.3±4.1 (26.4–52.1)	45.5±6.7[b] (38.5–61.3)	44.2±3.1[b] (34.2–52.8)	43.3±2.8[b] (33.8–51.4)
CV/TV (%)	4.3±2.4 (1.4–11.0)	4.6±2.7 (0.8–9.4)	6.1±2.8 (1.9–17.2)	4.8±2.4 (0.8–14.6)
mCV/CV (%)	3.4±1.8 (0.8–5.0)	2.8±1.3 (0.2–7.6)	4.1±1.2 (1.8–6.1)	3.2±1.2 (1.8–5.7)

[a] Initial callus volume of the bone graft was 1.42 cm^3
[b] $P<0.05$ in comparison to the cage alone group (group 1)

earlier new bone formation, both within and around the cages, compared to the other groups. There were no significant differences in new bone matrix formation between groups 2, 3 and 4 after 6 weeks. In contrast to the other groups, all cages coated with BMP-2 (group 4) showed new bone formation around and within the cages at 9 weeks.

Discussion

The purpose of this study was to compare the efficacy and safety of combined IGF-I and TGF-ß1 application with BMP-2 application and autologous cancellous bone grafts at an early time point (12 weeks) in an in vivo sheep cervical spine interbody fusion model.

Regardless of the interbody fixation technique, the sheep cervical spine will normally reach solid fusion after 24 weeks. Therefore, it was not the purpose of this study to achieve solid bony fusion. The study aimed, instead, to analyse a developing "bony fusion" at an early time point (12 weeks), in order to determine subtle distinctions between the various fixation techniques.

It is well known that the surgical intervention, including the decortication of the endplates, seems, in itself, to stimulate intervertebral fusion to a limited degree [34, 37]. To compare this "spontaneous fusion potential" with a growth factor or cancellous bone graft stimulated fusion, the cage alone group (group 1) was included in this study. The growth factor groups, as well as the bone graft group, showed a fusion potential significantly higher than the "spontaneous fusion potential" of the cage alone group.

BMP-2 demonstrated significant acceleration of interbody fusion in this study. In comparison to the cage alone group (group 1), representing the spontaneous fusion potential, the cage plus BMP-2 group (group 3) demonstrated a higher fusion rate in radiographic findings, a higher biomechanical stability, an advanced interbody fusion in histomorphometrical analysis, and an accelerated interbody fusion on fluorochrome sequence labelling.

These results are in concordance with previous animal studies using BMP-2 in experimental spinal fusion [4, 5, 6, 7, 8, 11, 14, 26, 27, 33, 34, 45]. However, the majority of these animal studies used BMP-2 in an intertransverse process fusion model of the lumbar spine [5, 7, 8, 16, 26, 27, 33]. Compared with these models, the biological environment of anterior interbody fusion evaluated in this study provides greater access to cancellous bone and bone marrow elements with osteogenetic potency. This favourable biological environment may, therefore, reduce the

Table 8 After 12 weeks, fluorochrome analysis was performed. Depicted are the number of fusion sites (of the different groups at different time points) at which the fluorochrome marker was present adjacent or within the cage or bone graft

Indices	Group 1 (*n*=8) Cage		Group 2 (*n*=8) Cage+bone graft		Group 3 (*n*=8) Cage+BMP-2		Group 4 (*n*=8) Cage+IGF-I+TGF-ß1	
	Adjacent	Within	Adjacent	Within	Adjacent	Within	Adjacent	Within
3 weeks	1	1	0	1	2	2	2	2
6 weeks	1	5	4	6	3	4	4	4
9 weeks	1	6	4	6	8	8	6	6

amount of growth factors needed to promote fusion, making the use of growth factors more economically feasible. In either case, it should be remembered that the dose of growth factors may need to be adjusted to the different biological environments and to different carriers. In several studies using an intertransverse fusion model and a collagen carrier, high BMP-2 doses (up to 1500 µg) were necessary to achieve intertransverse fusion [5, 7, 8, 16, 26, 27, 33]. In this study, a small dose of BMP-2 (approximately 150 µg) applied with a PDLLA-coated cage was able to accelerate anterior interbody bone matrix formation significantly. Due to the local administration via a PDLLA coating of cages, high and continuously released BMP-2 concentrations can be obtained at the fusion site [35]. The quantity and the release of the incorporated growth factors is small in relation to the total organism. Therefore, no systemic side effects on blood count, electrolytes, glucose levels, thyroid hormones, body weight or body temperature caused by BMP-2 were observed in this study.

Besides that, BMP-2 is also able to induce de novo bone in ectopic soft tissue sites, even in the absence of bone marrow elements [2, 46]. Some authors think that a growth factor is required to induce ectopic bone to promote interbody fusion sufficiently [4, 26, 27]. However, this characteristic of BMP-2 might also be harmful. In previous studies, new bone formation induced in the ligamentum flavum by BMP-2 resulted in flattening of the spinal cord [28]. Hoshi and co-workers [14] showed that BMP-2 induced ossification of the spinal ligaments, resulting even in spinal cord compression. Other authors have suggested that BMP-2 might play an important role in the ossification of spinal ligaments, especially the posterior longitudinal ligament [14, 22]. In this study, anterior interbody discectomy and fusion was performed, preserving the posterior longitudinal ligament. In concordance with Zdeblick et al. [45] and Hecht et al. [11], also using an anterior interbody fusion model, we were not able to determine any ossification of the posterior longitudinal ligament using BMP-2 applied by a PDLLA-coated interbody cage.

In previous anterior interbody fusion models using BMP-2, threaded cages were applied to stabilise the anterior spinal column [4, 6, 11, 45]. Zdeblick and co-workers [45], for example, used BAK-cages in a three-level fusion model in the cervical spine of the goat. In in vitro experiments using sheep cervical spines, Kandziora and co-workers [19] showed profound biomechanical differences between threaded cages and cylindrical cages like the meshed titanium cage used in this study. Especially in bending, the vertical cylinder design cages showed significantly higher stiffness and lower range of motion than the threaded cages. Nevertheless, no relevant differences in interbody bone matrix formation could be observed comparing the results of this study using BMP-2 plus cylindrical cages with previous studies using BMP-2 plus threaded cages [4, 6, 11, 45]. This suggests that the osteoinductive effect of BMP-2 applied in the intervertebral space is, to a certain extent, independent from the biomechanical properties of the intervertebral implant.

In contrast to BMP-2, IGF-I and TGF-ß1 are not able to induce de novo bone growth in ectopic soft tissue sites [24]. IGF-I stimulates the replication of osteoblasts and the synthesis of bone matrix [13]. TGF-ß1 regulates different cell types that are directly involved in bone remodeling and bone matrix formation, such as mesenchymal cells, chondrocytes, osteoblasts, and osteoclasts [31, 32]. Due to these characteristics, both growth factors have demonstrated their ability to accelerate the spontaneous fusion potential [9].

In this study the combined application of IGF-I and TGF-ß1 was able to promote interbody bone matrix formation. In comparison to the cage alone group (group 1) the cage plus IGF-I and TGF-ß1 group (group 4) demonstrated a significantly higher fusion rate in radiographic findings, a higher biomechanical stability, a more advanced interbody fusion in histomorphometrical analysis, and an accelerated interbody fusion on fluorochrome sequence labelling.

These results are in concordance with previous animal studies using IGF-I and TGF-ß1 in other anatomical locations. In these studies, IGF-I and TGF-ß1 individually and in combination have been shown to improve bone matrix formation and bone remodelling by direct and indirect mechanisms [3, 9, 13, 25, 29, 32, 40].

For systemically applied IGF-I and TGF-ß1, side effects have been described depending on concentration [25, 39, 43]. In particular, effects on electrolytes, glucose levels and thyroid hormones have been specified [39, 43]. However, based on these preliminary results, no systemic side effects on blood count, electrolytes, glucose levels, thyroid hormones, body weight or body temperature caused by IGF-I or TGF-ß1 were observed in this study. This may be due to the local administration of IGF-I and

TGF-ß1 via a PDLLA-coating of cages providing high local but low systemic concentrations of the growth factors [35].

One aim of this study was to compare autologous bone grafts (group 1) with growth factors (groups 3 and 4) in experimental spinal fusion. In comparison to the bone graft group, both growth factor groups (BMP-2 and combined IGF-I/TGF-ß1) showed significantly lower residual motion on functional radiographic evaluation, higher bone mineral density of the callus and higher biomechanical stability in extension, rotation and bending after 12 weeks in vivo. Additionally, although there was no significant difference between the bone graft group and the growth factor groups in the total amount of bone in the intervertebral space (bony callus volume and bone volume/total volume ratio) after 12 weeks, the fact that in group 2 the cages were initially filled with 1.42 cm^3 of cancellous bone must be taken into consideration. Therefore, the amount of new-formed bone in the bone graft group was approximately 4 cm^3 (range 5.4–1.42 cm^3; see Table 3). In contrast, the two growth factor groups showed an amount of new formed bone of 5.4 cm^3 (BMP-2) and 5.2 cm^3 (IGF-I/TGF-ß1), respectively (Table 3). This is the first study to describe an acceleration of intervertebral bone matrix formation with combined IGF-I/TGF-ß1 application in comparison to autologous cancellous bone grafts. However, this effect has already been described for BMP-2 [5, 8, 16, 26, 27, 45]. Boden et al. [5] and Zdeblick et al. [45], for example, have demonstrated that in comparison to autologous cancellous bone grafts BMP-2 was able to accelerate spinal fusion.

Another aim of this study was to compare IGF-I and TGF-ß1 with BMP-2 application in the sheep cervical spine fusion model. At present, the "ideal" concentrations for any growth factors to induce fusion are unknown. Some studies on BMP-2 have demonstrated good results in anterior spinal fusion models with BMP-2 doses ranging between 100 and 250 µg [4, 11, 45]. In other studies, the 5:1 ratio of IGF-I and TGF-ß1 applied by a PDLLA-coated implant has proven to be most effective [20, 36]. Therefore, comparable concentrations of BMP-2 (150 µg) and IGF-I (150 µg)/TGF-ß1 (30 µg) were chosen for this study. Using these concentrations, only slight and inconsistent differences could be evaluated comparing the two growth factor groups. The BMP-2 group showed significantly lower residual motion on functional radiographic evaluation and higher intervertebral bone matrix formation on fluorochrome sequence labelling at 9 weeks in comparison to the IGF-I/TGF-ß1 group. This slightly higher amount of bone matrix in the intervertebral space of the BMP-2 group might be due to the specific characteristic of BMP-2 of inducing de novo bone [2, 46]. However, this new formed bone did not result in a higher biomechanical stability of the BMP-2 fused motion segments. In contrast, the IGF-I/TGF-ß1 group showed a significantly higher bone mineral density of the callus than the BMP-2 group. This effect might be correlated with the ability of IGF-I and TGF-ß1 to accelerate bone remodelling, by influencing osteoblasts and osteoclasts [3, 25, 29, 32]. However, this effect was also not correlated with a higher biomechanical stability of the fused motion segments. Beside that, no relevant differences could be evaluated between the BMP-2 and the IGF-I/TGF-ß1 group in radiographic, biomechanical, or histological results. On the basis of these preliminary results, no systemic side effects of either growth factor could be determined.

Conclusion

In comparison to the cage alone group, the local application of growth factors (BMP-2 or combined IGF-I/TGF-ß1) significantly improved results of interbody bone matrix formation in this sheep cervical spine fusion model. In comparison to the cancellous bone graft group, both growth factors significantly improved the biomechanical results of interbody fusion. An additional advantage of the use of growth factors is that donorsite morbidity of the iliac crest graft can be avoided. On the basis of the preliminary results of this study, the combined IGF-I/TGF-ß1 application yields equivalent results to BMP-2 application at an early time point in this anterior sheep cervical spine fusion model. Therefore, combined IGF-I/TGF-ß1 application has proven to have considerable effectiveness in experimental spinal fusion. Although no systemic side effects were observed for either growth factor during the 12-week follow-up period, long-term effects of these growth factors are still unknown. Further, in particular long-term, comparative in vivo studies of different growth factors might elucidate which growth factor or combination of growth factors – also taking into account those not yet tested – could create the basis for spine fusion at an optimised velocity and with minimised risk.

References

1. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R (2000) Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and oestrogen deficiency. J Bone Miner Res 15:683–690
2. Aspenberg P, Turek T (1996) BMP-2 for intramuscular bone induction. Effect in squirrel monkeys is dependent on implantation site. Acta Orthop Scand 67:3–6
3. Beck L, Amento E, Xu Y, Deguzman L, Lee W, Nguyen T, Gillet N (1993) TGF-beta 1 induces bone closure of skull defects – temporal dynamics of bone formation in defects exposed to rhTGF-beta 1. J Bone Miner Res 8: 753–761

4. Boden SD, Martin GJ Jr, Horton WC, Truss TL, Sandhu HS (1998) Laproscopic anterior spinal arthrodesis with rhBMP-2 in a titanium interbody threaded cage. J Spinal Disord 11:95–101
5. Boden SD, Martin GJ Jr, Morone MA, Ugbo JL, Moskovitz PA (1999) Posterolateral lumbar intertransverse process spine arthrodesis with recombinant human bone morphogenetic protein 2/hydroxyapatite-tricalcium phosphate after laminectomy in the nonhuman primate. Spine 24:1179–1185
6. Boden SD, Zdeblick TA, Sandhu HS, Heim SE (2000) The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine 25:376–381
7. David SM, Gruber HE, Mayer RA Jr, Murakami T, Tabor OB, Howard BA, Wozney JM, Hanley EN Jr (1999) Lumbar spinal fusion using recombinant human bone morphogenetic protein in the canine. A comparison of three dosages and two carriers. Spine 24:1973–1979
8. Fischgrund JS, James SB, Chabot MC, Hankin R, Herkowitz HN, Wozney JM, Shirkhoda A (1997) Augmentation of autograft using rhBMP-2 and different carrier media in the canine spinal fusion model. J Spinal Disord 10:467–472
9. Fujimoto A, Tanizawa T, Nishida S, Yamamoto N, Soshi S, Endo N, Takahashi HE (1999) Local effects of transforming growth factor-beta 1 on rat calvaria: changes depending on the dose and the injection site. J Bone Miner Metab 17:11–17
10. Grauer JN, Patel TC, Erulkar JS, Troiano NW, Panjabi MM, Friedlaender GE (2001) 2000 Young Investigator Research Award winner. Evaluation of OP-1 as a graft substitute for intertransverse process lumbar fusion. Spine 26:127–133
11. Hecht BP, Fischgrund JS, Herkowitz HN, Penman L, Toth JM, Shirkhoda A (1999) The use of recombinant human bone morphogenetic protein 2 (rhBMP-2) to promote spinal fusion in a nonhuman primate anterior interbody fusion model. Spine 24:629–636
12. Hermann R, Schmidmaier G, Markl B, Resch A, Hahnel I, Stemberger A, Alt E (1999) Antithrombogenic coating of stents using a biodegradable drug delivery technology. Thromb Haemost 82:51–57
13. Hock J, Centrella M, Canalis E (1988) Insulin like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology 122:254–260
14. Hoshi K, Amizuka N, Sakou T, Kurokawa T, Ozawa H (1997) Fibroblasts of spinal ligaments pathologically differentiate into chondrocytes induced by recombinant human bone morphogenetic protein-2: morphological examinations for ossification of spinal ligaments. Bone 21:155–162
15. Isgaard J, Nilson A, Lindahl A, Jansson J, Isaksson O (1986) Effects of local administration of GH and IGF-I on longitudinal bone growth in rats. Am J Physiol 250:367–372
16. Itoh H, Ebara S, Kamimura M, Tateiwa Y, Kinoshita T, Yuzawa Y, Takaoka K (1999) Experimental spinal fusion with use of recombinant human bone morphogenetic protein 2. Spine 24:1402–1405
17. Kandziora F, Kerschbaumer F, Starker M, Mittlmeier T (2000) Biomechanical assessment of transoral plate fixation for atlantoaxial instability. Spine 25: 1555–1561
18. Kandziora F, Pflugmacher R, Scholz M, Schnake K, Schröder R, Mittlmeier T (2001) Comparison between sheep and human cervical spines: an anatomic, radiographic, bone mineral density, and biomechanical study. Spine 26:1028–1037
19. Kandziora F, Pflugmacher R, Schäfer J, Duda G, Haas NP, Mittlmeier T (2001) Biomechanical comparison of cervical spine interbody fusion cages. Spine 26:1850–1857
20. Kandziora F, Schmidmaier G, Schollmeier G, Bail H, Pflugmacher R, Görke T, Wagner M, Raschke M, Mittlmeier T, Haas NP (2002) IGF-I and TGF-ß1 application by a poly-(D,L-lactide) coated interbody cage promotes fusion in the sheep cervical spine. Spine 27 (in press)
21. Kleemann R (1999) Entwicklung eines Wirbelsäulenprüfstands zur Testung von Implantaten an der Halswirbelsäule. Semesterarbeit. Fakultät für Maschinenbau, Technische Universität Berlin
22. Kon T, Yamazaki M, Tagawa M, Goto S, Terakado A, Moriya H, Fujimura S (1997) Bone morphogenetic protein-2 stimulates differentiation of cultured spinal ligament cells from patients with ossification of the posterior longitudinal ligament. Calcif Tissue Int 60:291–296
23. Laursen M, Hoy K, Hansen ES, Gelineck J, Christensen FB, Bunger CE (1999) Recombinant bone morphogenetic protein-7 as an intracorporal bone growth stimulator in unstable thoracolumbar burst fractures in humans: preliminary results. Eur Spine J 8:485–490
24. Lind M (1998) Growth factor stimulation on bone healing. Effects on osteoblasts, osteotomies, and implants fixations. Acta Orthop Scand Suppl 283: 2–37
25. Lind M, Schuhmacker B, Soballe K, Keller J, Melson F, Bunger C (1993) Transforming growth factor-beta enhances fracture healing in rabbit tibiae. Acta Orthop Scand 64:553–556
26. Martin GJ Jr, Boden SD, Marone MA, Marone MA, Moskovitz PA (1999) Posterolateral intertransverse process spinal arthrodesis with rhBMP-2 in a nonhuman primate: important lessons learned regarding dose, carrier, and safety. J Spinal Disord 12:179–186
27. Meyer RA Jr, Gruber HE, Howard BA, Tabor OB Jr, Murakami T, Kwiatkowski TC, Wozney JM, Hanley EN Jr (1999) Safety of recombinant human bone morphogenetic protein-2 after spinal laminectomy in the dog. Spine 24:747–754
28. Mimatsu K, Kishi S, Hashizume Y (1997) Experimental chronic compression on the spinal cord of the rabbit by ectopic bone formation in the ligamantum flavum with bone morphogenetic protein. Spinal Cord 35:740–746
29. Mohan S, Baylink D (1991) Bone growth factors. Clin Orthop 263:30–48
30. Nielson H, Isgaard J, Lindahl A, Peterson L, Isaksson O (1987) Effects of unilateral arterial infusion of GH and IGF-I on tibial longitudinal bone growth in hypophysectomized rats. Calcif Tissue Int 40:91–96
31. Pfeilschifter J, Oechsner M, Naumann A, Gronwald R, Minne H, Ziegler R (1990) Stimulation of bone matrix apposition in vitro by local growth factors: a comparison between insulin-like growth factor I, platelet derived growth factor and transforming growth factor beta. Endocrinology 127:69–75
32. Roberts A, Sporn M, Bolander M (1990) Transforming growth factor-beta and the initiation of chondrogenesis in the rat femur. J Cell Biol 110: 2195–2207
33. Sandhu HS, Kanim LE, Kabo JM, Toth JM, Zeegen EN, Liu D, Delamater RB, Dawson EG (1996) Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion. Spine 21:2115–2122
34. Sandhu HS, Kanim LE, Toth JM, Kabo JM, Liu D, Delamarter RB, Dawson EG (1997) Experimental spinal fusion with recombinant human bone morphogenetic protein-2 without decortication of osseous elements. Spine 22: 1171–1180

35. Schmidmaier G, Wildemann B, Stemberger A, Haas NP, Raschke M (2001) Biodegradable poly-(D,L-lactide) coating of implants for continuous release of growth factors. J Biomed Mat Res 58:449–455
36. Schmidmaier G, Wildemann B, Stemberger A, Haas NP, Raschke M (2001) Local application of growth factors (insulin-like growth factor-1 and transforming growth factor-beta1) from a biodegradable poly(D,L-lactide) coating of osteosynthetic implants accelerates fracture healing in rats. Bone 28: 341–350
37. Slappey G, Toribatake Y, Ganey TM, Odgen JA, Hutton WC (1998) Guidelines to decortication in posterolateral spine fusion. J Spinal Disord 11:102–109
38. Steinbrech DS, Mehrara BJ, Rowe NM, Dudziak ME, Luchs JS, Saadeh PB, Gittes GK, Longaker MT (2000) Gene expression of TGF-beta, TGF-beta receptor, and extracellular matrix proteins during membranous bone healing in rats. Plast Reconstr Surg 105:2028–2038
39. Terrell TG, Working PK, Chow CP, Green JD (1993) Pathology of recombinant human transforming growth factor-beta 1 in rats and rabbits. Int Rev Exp Pathol 34B:43–67
40. Thaller S, Hoyt J, Tesluck H, Holmes R (1993) The effect of insulin growth factor-I on calvarial sutures in Sprague-Dawley rat. J Craniofac Surg 4:35–39
41. Trippel S, Coutts R, Einhorn T, Mundy R, Rosenfeld R (1996) Growth factors as therapeutic agents. J Bone Joint Surg Am 78:1272–1286
42. Urist MR (1965) Bone: formation by autoinduction. Science 150:893–899
43. Wilton P (1992) Treatment with recombinant human insulin-like growth factor I of children with growth hormone receptor deficiency (Laron syndrome). Kabi Pharmacia Study Group on insulin-like growth factor I treatment in growth hormone insensitivity syndromes. Acta Paediatr Suppl 383: 137–142
44. Yamada Y, Harada A, Hosoi T, Miyauchi A, Ikeda K, Otha H, Shiraki M (2000) Association of transforming growth factor beta 1 genotype with therapeutic response to active vitamin D for postmenopausal osteoporosis. J Bone Miner Res 15:415–420
45. Zdeblick TS, Ganayem AJ, Rapoff AJ, Swain C, Bassett T, Cooke ME, Markel M (1998) Cervical interbody fusion cages. An animal model with and without bone morphogenetic protein. Spine 23:758–765
46. Zegzula HD, Buck DC, Brekke J, Wozney JM, Hollinger JO (1997) Bone formation with use of rhBMP-2 (recombinant human bone morphogenetic protein-2). J Bone Joint Surg Am 79: 1778–1790

Diese Untersuchungen konnten zeigen, dass die intervertebrale Spondylodese durch IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage bessere Ergebnisse zeigt als mit autologem Knochenmaterial. Gleichzeitig konnte die Entnahmemorbidität autologen Knochenmaterials vermieden werden. Zusätzlich konnte demonstriert werden, dass die intervertebrale Spondylodese durch IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage der Applikation von BMP-2 äquivalent ist.

3 Diskussion

Die Halswirbelsäule des Schafes wird häufig als Tiermodell der humanen Halswirbelsäule verwendet, obwohl nur wenige vergleichende quantitative Daten existieren [201,202]. Trotz eindeutiger anatomischer Unterschiede zwischen der humanen Halswirbelsäule und der Schafshalswirbelsäule, konnte in diesen Untersuchungen [92] erstmalig eine gute Analogie zwischen den beiden Spezies hinsichtlich der Bandscheibenraumhöhe, funktionsradiologischer Parameter, der Knochendichte und den biomechanischen Parametern Steifigkeit, Bewegungsumfang, neutrale und elastische Zone nachgewiesen werden. Auf der Basis dieser Ergebnisse sind besonders die tierexperimentellen Daten für die funktionsradiologischen Untersuchungen, die Implantatsinterung, die entscheidend von der Knochendichte der angrenzenden Wirbelkörper abhängt, und die biomechanischen Parameter gut auf die humane Situation übertragbar. Speziell die Übereinstimmungen zwischen den Spezies für das Bewegungssegmente C3/4 machte die Evaluation dieses Bewegungssegmentes für die weiteren Untersuchungen sinnvoll.

Ein Beobachtungszeitraum von 12 Wochen wurde in diesen Untersuchungen gewählt, da zu diesem Zeitpunkt die Spondylodese im Schaf weit fortgeschritten, jedoch noch nicht vollständig ist [39,163]. Daher sind in dieser frühen Phase der Spondylodese besonders gut radiologische, biomechanische und histologische Unterschiede im Einheilungsverhalten der Implantate zu demonstrieren [39].

3.1 Einfluss des Cagedesigns auf die intervertebrale Spondylodese

3.1.1 Zusammenhang zwischen Primär- und Sekundärstabilität von Cages

Die Primärstabilität eines Spongiosa-augmentierten Cages resultiert fast ausschließlich aus den mechanischen Eigenschaften des Cages [87,93]. Erst sekundär, durch die Ausbildung der intervertebralen Knochenmatrix, trägt die inkorporierte Spongiosa zur Gesamtstabilität des Cage-Spongiosa-Komplexes im Intervertebralraum bei [200]. Die in dieser Untersuchung durchgeführte biomechanische in vitro Studie [93] zeigte erstmalig designabhängige Unterschiede in der Primärstabilität der Cagegruppen. So konnte gezeigt werden, dass die Primärstabilität von Box- und Zylinderdesign Cages signifikant größer war, als die

von Schraubendesign-Cages. Die signifikant größte Steifigkeit in allen Bewegungsrichtungen wies der Syncage-C (Boxdesign) auf. Im Gegensatz dazu zeigte der Harmscage (Zylinderdesign) eine signifikant geringere Primärstabilität. Für den Syncage-C konnte in vitro eine signifikant höhere Primärstabilität als für den trikortikalen Beckenkammspan nachgewiesen werden, während für den Harmscage ähnliche Primärstabilitätswerte evaluiert wurden.

Diese biomechanischen in vitro Ergebnisse der Cage Evaluation [93] stehen jedoch in eindeutigem Gegensatz zu den in vivo Ergebnissen dieser Untersuchung [96]. Während der Beckenkammspan und der Harmscage die in vitro Steifigkeit in vivo signifikant steigern konnten, bestand zwischen in vitro und in vivo Steifigkeit des Syncage-C kein signifikanter Unterschied. Diese Ergebnisse stehen auch im Gegensatz zu bisherigen Postulaten, nach denen eine hohe Primärstabilität auch zu einer hohen Sekundärstabilität während der Einheilung der Implantate führt [21,87,102,117,136,153,186,198]. Die deutlichen Unterschiede zwischen den biomechanischen in vitro und in vivo Ergebnissen dieser Studie weisen vielmehr darauf hin, dass offensichtlich „biologisch-mechanische Qualitäten“ eines Cages existieren, die einen entscheidenden Einfluss auf die Sekundärstabilität haben und die durch reine biomechanische in vitro Tests, mit offensichtlich nur begrenzter Aussagekraft, bisher nicht zu determinieren sind.

3.1.2 Zusammenhang zwischen Auflagefläche und Sinterung von Cages

Zahlreiche klinische Studien konnten bei der intervertebralen Fusion mit autologem trikortikalen Beckenkammspanimplantaten eine signifikante Reduktion der Bandscheibenraumhöhe und eine Kyphosierung des Bewegungssegmentes im postoperativen Verlauf nachweisen [44,60,109,165]. Diese klinischen Beobachtungen konnten über einen Zeitraum von 12 Wochen auch in den vorliegenden tierexperimentellen Untersuchungen bestätigt werden.
Im Gegensatz dazu wurden intervertebrale Cages mit der Zielsetzung entwickelt, die Höhe des Intervertebralraums und die Lordose des Bewegungssegmentes während der knöchernen Fusion zu erhalten [200]. Jedoch liegt bisher zu diesem Themenkomplex nur eine tierexperimentelle in vivo Untersuchung vor. Sandhu [165] konnte in einem Wirbelsäulenfusionsmodell am Schaf nachweisen, dass Schraubendesign-Cages in der Lage sind, die postoperativ erzielte Distraktion und

Lordose besser zu erhalten als ein autologes trikortikales Beckenkammspanimplantat. Experimentelle Untersuchungen zu den in dieser Studie verwendeten Cagedesigns (Harmscage = Zylinderdesign; Syncage-C = Boxdesign) hinsichtlich deren Sinterungsverhalten wurden bisher nicht durchgeführt. Die Daten dieser Untersuchung zeigen, dass beide Cages und der trikortikale Beckenkammspan in der Lage waren, postoperativ eine Distraktion und Lordosierung des Bewegungssegmentes in gleichem Ausmaß zu erzielen. Jedoch kam es in allen Gruppen während der 12-wöchigen Nachbeobachtungszeit zu einer signifikanten Sinterung der Implantate, die dazu führte, dass die präoperative Bandscheibenraumhöhe deutlich unterschritten wurde. Zusätzlich zeigte sich in allen Gruppen eine Re-Kyphosierung des Bewegungssegmentes. Während die Reduktion der Bandscheibenraumhöhe und der Verlust der Lordose in beiden Cage-Gruppen Folge eines Einsinken des Cages in die benachbarten Endplatten war, resultierte die Sinterung und Re-Kyphosierung in der Beckenkammspan-Gruppe aus einem Kollaps des Implantats. Im Vergleich zum Beckenkammspan waren beide Cages jedoch in der Lage im 12 Wochen Verlauf die Bandscheibenraumhöhe und die Lordose signifikant besser zu erhalten. Obwohl der Syncage-C zum 2 Wochen Zeitpunkt eine geringere Sinterung und Re-Kyphosierung aufwies als der Harmscage, konnten im weiteren zeitlichen Verlauf keine Unterschiede zwischen den Cagedesigns bestimmt werden. Gemäß der häufig geäußerten Hypothese - je größer die Auflagefläche des Cages, desto geringer die Sinterung des Cages [51,79,165] – hätte jedoch der Syncage-C, dessen Auflagefläche mehr als doppelt so groß ist wie die des Harmscages [96], ein geringeres Sinterungsverhalten aufweisen müssen. Daher ist die oben genante Hypothese zu verwerfen. Die Auflagefläche eines Cages beeinflusst die Sinterung der Implantate nur geringfügig in der direkten postoperativen Phase, ohne jedoch einen Effekt auf den Endzustand des Sinterungsvorgangs (in dieser Studie 12 Wochen) zu haben.

3.1.3 Zusammenhang zwischen Design und Einheilungsverhalten von Cages

Zum Einheilungsverhalten der hier untersuchten Cagedesigns liegen bisher keine tierexperimentellen Studien vor. Jedoch wurden Schraubendesign-Cages intensiv in vivo untersucht [11,39,70,208]. So konnten mehrere Autoren ein ausreichendes Einheilen von Spongiosa-gefüllten schraubendesignartigen Cages in zahlreichen

Tiermodellen histologisch nachweisen [11,39,70,165,208].

Die histomorphometrische Analyse in dieser Studie 12 Wochen postoperativ zeigte eine signifikant höhere intervertebrale Knochen-/Gesamtvolumen Relation in der Harmscage-Gruppe als in der Beckenkammspan-Gruppe [98]. Dabei ist die ungünstige Knochen-/Gesamtvolumen-Relation des Knochenspanimplantats vorrangig auf dessen mechanisch induzierte Fragmentierung zurückzuführen. Kein Unterschied in der Knochen-/Gesamtvolumen-Relation konnte zwischen dem Syncage-C und dem Beckenkammspanimplantat evaluiert werden. Beim Vergleich beider Cage-Gruppen, die mit der selben Menge Spongiosa gefüllt wurden, zeigte sich auch kein Unterschied in der Konfiguration der Knochen/Implantat-Grenzfläche. Der Harmscage wies jedoch eine signifikant höhere intervertebrale Knochen-/Gesamtvolumen Relation auf als der Syncage-C, was einem akzelerierten Einheilungsverhalten des Harmscages entspricht [208].

Cages wurden unter der Vorstellung entwickelt, dass eine mechanische Protektion des inkorporierten autologen Knochenmaterials eine Einheilung des Implantats fördert [21,102,117]. Dabei wurde behauptet, dass ausschließlich die mechanische Stabilität und weniger das Design eines Cages von Bedeutung ist [95,136,186]. Kanayama [87] konnte jedoch in einer in vitro Untersuchung zeigen, dass das Cagedesign einen signifikanten Einfluss auf die Drucke hat, die innerhalb eines Cages auf die inkorporierte Spongiosa wirken. Er demonstrierte, dass die Größe der maximalen Pore eines Cages entscheidend für die Reduktion der Stress-Protektion der inkorporierten Spongiosa ist. Dabei formulierte er folgende, auf dem Wolf´schen Gesetz basierende Hypothese: „Je größer die maximale Pore in der Auflagefläche eines Cages, desto geringer das „stress shielding“ auf die inkorporierte Spongiosa und desto günstiger das Einheilungsverhalten des Cages“ [87]. Eine weitere Hypothese wurde erstmalig im Rahmen dieser Untersuchungen entworfen [93]: Der Cage und die inkorporierte Spongiosa konkurrieren um das Volumen des Intervertebralraums. Je mehr Spongiosa im Intervertebralraum inkorporiert werden kann, desto wahrscheinlicher kommt es zur knöchernen Einheilung des Implantats [200]. Demzufolge müsste der Cage, der bei geringstem Volumen die größten Primärstabilitätswerte aufweist, ein optimiertes Milieu für die Einheilung des Implantats erreichen. Daher wurde die sogenannte Volumen-bezogene-Steifigkeit definiert, die die Primärstabilität des Cages in Relation zu dessen Volumen beschreibt. Diese Hypothesen zur Volumen-bezogenen-Steifigkeit und zum „stress

shielding“ können die oben genanten Unterschiede in der intervertebralen Knochen-/Gesamtvolumen Relation beider Cages erklären. Der Harmscage besitzt eine höhere Volumen-bezogene-Steifigkeit und eine ca. doppelt so große maximale Pore und demzufolge einen deutlich höheren Intra-Cage-Kompressions-Druck als der Syncage-C. Daher ist das „stress shielding“ der inkorporierten Spongiosa im Harmscage deutlich reduziert. Diese Hypothesen erklären auch die histomorphologisch evaluierte höhere Osteoklastenaktivität innerhalb des Syncage-C. Durch die geringere Volumen-bezogene-Steifigkeit des Implantats und die mechanische Stress-Protektion der Spongiosa innerhalb des Syncage-C wird die nicht druckbelastete Spongiosa abgebaut. Demzufolge muß konstatiert werden, dass das "stress shielding" innerhalb eines Cages und die Volumen-bezogene-Steifigkeit eines Cages, im Vergleich zu anderen Parametern, eine dominierende Rolle für das Einheilungsverhalten eines Cages haben.

3.2 Einfluss des Carrier-Systems auf die intervertebrale Spondylodese

Die optimale Methode zur lokalen Applikation von Wachstumsfaktoren in der Wirbelsäulenchirurgie wird immer noch kontrovers diskutiert. Derzeit befinden sich vorwiegend Kollagenschwämme in experimenteller und zum Teil auch klinischer Anwendung [11,12,13,14,53,70,84,127,131,163,164,208,210]. Jedoch ist die Sicherheit und die Freisetzungskinetik dieser Carrier fragwürdig [127,180,184]. So wurden eine schnelle und unkontrollierte Freisetzung von Wachstumsfaktoren aus dem Kollagen-Carrier [127], Probleme bei der intraoperativen Platzierung [5,81,106,133], schlechte Kompressionseigenschaften [127] sowie eine fragliche Sicherheit des aus bovinem Material bestehendem Carriers hinsichtlich der Auslösung allergischer Reaktionen und der Übertragung von Infektionskrankheiten beschrieben [184].

Ein ideales Carrier-System sollte kontrolliert applizierbar sein und vor allem eine kontinuierliche, definierte und lokale Applikation von Wachstumsfaktoren gewährleisten und dabei ohne Nebenwirkungen vollständig resorbierbar sein [16,58,59,80]. Speziell die kontinuierliche Applikation der Wachstumsfaktoren ist dabei von entscheidender Bedeutung, da die Halbwertszeit der Wachstumsfaktoren im Minutenbereich liegt. Vor kurzem wurde eine biodegradierbare Poly-(D,L-laktid)-(PDLLA) Beschichtung zur Applikation bioaktiver Substanzen vorgestellt,

die diese Anforderungen erfüllen könnte [73,171]. Dieses Beschichtungssystem ist mechanisch stabil, vollständig degradierbar, erlaubt die lokale und kontinuierliche Freisetzung von Wachstumsfaktoren und ermöglicht die Beschichtung etablierter Wirbelsäulenimplantate mit bioaktiven Substanzen [172]. Ziel dieser Untersuchung war es unter anderem, die Wertigkeit dieses Carriers bei intervertebraler Applikation zu überprüfen.

Derzeit sind Poly-Laktidsäuren (PLA) und Poly-Glykolidsäuren (PGA) und deren Kopolymere als biodegradierbare Implantate und Carrier-Systeme etabliert [41,42,53,82,107,114,161,163]. In vorangegangenen Untersuchungen, besonders bei Verwendung von Poly-Laktidsäuren und Poly-Glykolidsäuren [53,109,132,187] konnte gezeigt werden, dass Degradationsprodukte von Polymeren zur Alteration der biologischen Umgebungsbedingungen führen können [80,114,128]. Speziell die Ausbildung von Osteolysen wurde beschrieben [53]. Um den Effekt der PDLLA Beschichtung auf die intervertebrale Fusion zu dokumentieren, wurden in dieser Untersuchung beschichtete und unbeschichtete Cages verglichen. Die Untersuchung der Laborparameter, des Körpergewichts und der Körpertemperatur zeigte keinen Unterschied zwischen den beiden Gruppen. Die histologische Evaluation konnte keine Osteolysen oder Entzündungsreaktionen in der PDLLA-Gruppe nachweisen. Auch die Anzahl der Makrophagen bzw. Fremdkörperriesenzellen in der histomorphologischen Untersuchung war in beiden Gruppen gleich. In vorangegangenen Untersuchungen [172] konnte jedoch darüber hinaus ein signifikant positiver Effekt der PDLLA-Beschichtung auf die Ausbildung der Knochenmatrix gezeigt werden. Im Vergleich zu den unbeschichteten Cages zeigten die PDLLA-beschichteten Cages eine signifikant höhere Knochendichte des Kallus und ein signifikant höheres Knochenvolumen/Gesamtvolumen-Verhältnis. Des weiteren konnten für die PDLLA- beschichteten Cages geringgradig bessere Werte für die Funktions-Röntgenuntersuchung, den Mineralsalzgehalt, das Kallusvolumen und die biomechanischen Parameter erhoben werden. Zusammenfassend läßt sich demzufolge festhalten, dass keine negativen Effekte auf die intervertebrale Fusion von der PDLLA-Beschichtung ausgehen, jedoch die positiven Ergebnisse früherer Untersuchungen nur tendenziell nachweisbar waren [171,172]. Die Ursache für die gering positiven Effekte der PDLLA-Beschichtung ist unklar, jedoch ist eine inflammatorische Reaktion im Rahmen der Degradation des PDLLA wahrscheinlich [171].

Darüber hinaus war es ein Ziel dieser Untersuchungen, erstmalig die Effektivität des PDLLA-Carriers im Vergleich zum Kollagen-Carrier zu evaluieren. Hierzu wurden beide Carrier-Systeme, versetzt mit analogen Mengen BMP-2, verglichen. Diese Untersuchungen ergänzen diesbezüglich vorangegangene Studien. Fischgrund [53] konnte z. B. keinen Unterschied zwischen Kollagenschwamm-Carriern und PGA nachweisen. Jedoch erzielten Zegzula [210] und Ozuna [141] bei Verwendung eines Poly-(D,L-laktide-coglykolide) (PLA/PGA)-Carriers in einem intertransversalen Wirbelsäulenfusionsmodell bessere Ergebnisse als bei Verwendung eines Kollagen-Carriers. Im Vergleich zur BMP-2 Applikation mittels Kollagen-Carrier zeigte in dieser Untersuchung die BMP-2 Applikation mittels PDLLA-Carrier ein signifikant höheres knöchernes Kallusvolumen und ein signifikant höheres Knochenvolumen / Gesamtvolumen Verhältnis. Auf der Basis der vorliegenden Untersuchungsergebnisse scheint daher die kontinuierliche Freisetzung von Wachstumsfaktoren aus dem PDLLA-Carrier der kurzfristigen Freisetzung aus dem Kollagen-Carrier überlegen.

3.3 Einfluss von Wachstumsfaktoren auf die intervertebrale Spondylodese

In der Wirbelsäulenchirurgie liegt die Zielsetzung der Wachstumsfaktorenapplikation in der Stimulation der intervertebralen Spondylodese [12,163]. Dadurch soll die Fusion beschleunigt, die Pseudarthroserate und Komplikationsrate gesenkt, die Rehabilitation der Patienten forciert und somit die Kosten der Behandlung reduziert werden [14]. Zusätzlich soll durch die Wachstumsfaktorenapplikation die Notwendigkeit zur Entnahme autologen Knochenmaterials und die damit verbundene Entnahmemorbidität vermieden werden [42,53,61]. Die Entnahmemorbidität des autologen Knochenmaterials stellt dabei ein zentrales Problem der Wirbelsäulenchirurgie dar. Erste Versuche durch die Applikation von Wachstumsfaktoren wie BMP-2 und BMP-7 mittels eines Kollagen-Carriers diese Entnahmemorbidität zu eliminieren waren zwar partiell erfolgreich, brachten jedoch andere Probleme mit sich. Neben den oben beschriebenen Problemen mit dem Kollagen-Carrier ist speziell die spinale Applikation der BMPs mit Nachteilen verbunden [81,106,133]. BMP-2 und BMP-7 sind in der Lage, eine de novo Synthese von Knochen in ektopischen Weichteilgewebe sogar in Abwesenheit von Knochenmarkelementen hervorzurufen [5,210]. In früheren Untersuchungen konnte

in Folge dessen gezeigt werden, dass BMP-2 induzierte Ossifikationen im Lig. flavum oder im Lig. longitudinale posterior [81,133] zu einer neurologisch wirksamen Myelonkompression führen können. Zusätzlich kommt BMP-2 pathophysiologisch eine entscheidende Bedeutung bei der hereditären OPLL (ossification of the posterior longitudinal ligament) zu, die mit einer Myelopathie einhergeht [81,106]. Daher besteht bei spinaler Applikation von BMPs die potentielle Gefahr durch eine überschießende Knochenneubildung eine Spinalkanalstenose zu induzieren.

Im Gegensatz zu BMP-2 sind IGF-I und TGF-β1 nicht zur de novo Synthese von Knochen in der Lage [118]. Vielmehr liegt die Wirksamkeit dieser Wachstumsfaktoren in der Förderung des spontanen Knochenbildungspotentials [57]. IGF-I und TGF-β1 regulieren dabei teilweise synergistisch eine Vielzahl unterschiedlicher Zellen wie Osteoblasten, Osteoklasten und Chondrozyten, die direkt an der Ossifikation, an der Ausbildung der Knochenmatrix und am Remodeling des Knochens beteiligt sind [147,157]. Eine ektopische Ausbildung von Knochen, zum Beispiel in spinalen Ligamenten, ist aufgrund dieses Wirkungsmechanismus nahezu ausgeschlossen und macht damit die Evaluation dieser Wachstumsfaktorenkombination in einem Wirbelsäulenfusionsmodell interessant.

Die Effektivität einer interkorporellen Spondylodese durch IGF-I und TGF-β1 appliziert mittels eines Poly (D,L-laktid) beschichteten Cage musste an Standard-Therapieverfahren gemessen werden. Als Standard-Therapieverfahren zur ventralen interkorporellen Spondylodese der Halswirbelsäule werden die Stabilisierung mit trikortikalem Beckenkammspan und mittels Spongiosa-augmentierter intervertebraler Cages angesehen. Zusätzlich hat sich die Applikation des Wachstumsfaktors BMP-2 zur Stimulation der intervertebralen Spondylodese als experimenteller Standard etabliert.

Zunächst wurde in einer Dosisfindungsstudie erstmalig die kombinierte Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage evaluiert. Dabei zeigten die mittlere und hohe Dosis von IGF-I und TGF-β1 einen signifikanten osteoinduktiven Effekt. Speziell die mittlere Dosis von 150 µg IGF-I plus 30 µg TGF-β1 wies dabei die größte Effektivität auf, da bei geringeren Dosen der osteoinduktive Effekt ausblieb und bei höheren Dosen keine Steigerung der

Knochenmatrixbildung induziert werden konnte. Zusätzlich waren für alle IGF-I und TGF-β1 Dosierungen keine unerwünschten Wirkungen auf die Laborparameter, das Körpergewicht oder die Körpertemperatur nachweisbar.

Zahlreiche Untersuchungen haben gezeigt, dass alleine die Dekortikation der Endplatten im Tiermodel eine Spondylodese induzieren kann [164,177]. Um dieses „spontane Fusionspotential“ von der Wirkung der Wachstumsfaktoren abzugrenzen, wurde die „Nur-Cage“-Gruppe den Wachstumsfaktoren-Gruppen gegenübergestellt. Dabei zeigten die Wachstumsfaktoren-Gruppen in allen Bereichen signifikant bessere Fusionsparameter.

Die kombinierte Applikation von IGF-I und TGF-β1 führte außerdem zu einer Beschleunigung der intervertebralen Fusion. Im Vergleich zur „Nur-Cage“-Gruppe zeigten IGF-I und TGF-β1 eine höhere Fusionsrate in den radiologischen Parametern, eine höhere biomechanische Stabilität und eine fortgeschrittene intervertebrale Fusion in den histologischen Untersuchungen. Diese Resultate stehen im Einklang mit früheren tierexperimentellen Untersuchungen, die die Wirksamkeit von IGF-I und TGF-β1 in anderen anatomischen Lokalisationen untersuchten [9,57,78,118,119,134,157,188]. In vorangegangenen Untersuchungen konnten aber bei der systemischen Applikation von IGF-I oder TGF-β1 dosisabhängige Nebenwirkungen beobachtet werden [118,187,204]. Im Einzelnen waren Elektrolytstörungen, Störungen des Glukosestoffwechsels und der Schilddrüsenhormonkonzentrationen nachweisbar [187,204]. In den vorliegenden Untersuchungen konnten diese unerwünschten Wirkungen bisher jedoch nicht nachvollzogen werden. Dieses Ausbleiben unerwünschter Wirkungen läßt sich vorwiegend auf die lokale Applikation der Wachstumsfaktoren durch die PDLLA-Beschichtung der Implantate zurückführen, die eine hohe lokale aber sehr geringe systemische Konzentration der Wachstumsfaktoren gewährleistet [172].

Die Applikation von BMP-2 führte in diesen Untersuchungen konstant zu einer Beschleunigung der intervertebralen Fusion. Im Vergleich zur „Nur-Cage“-Gruppe zeigte sich bei Verwendung von BMP-2 eine höhere Fusionsrate in den radiologischen Parametern, eine höhere biomechanische Stabilität, und eine fortgeschrittene intervertebrale Fusion in den histologischen Untersuchungen. Diese Resultate stehen im Einklang mit früheren Untersuchungen [11,12,13,14,42,53,70,84,127,131,163,164,208,210]. Die meisten dieser Ergebnisse

wurden jedoch in dorsalen intertransversalen Fusionsmodellen der Lendenwirbelsäule evaluiert [12,42,53,84,127,131,163]. In diesen Fusionsmodellen waren BMP-2 Dosen von bis zu 1500 µg notwendig, um eine Knochenneubildung zu induzieren [12,42,53,84,127,131,163]. Im Vergleich hierzu bietet das verwendete ventrale zervikale Fusionsmodell der Schafswirbelsäule einen verbesserten Zugang zu spongiösem Knochen und osteogenetisch potenten Knochenmarkszellen. In diesen Studien waren demzufolge 10-fach geringere BMP-2 Dosen (150 µg) ausreichend, um einen signifikanten osteoinduktiven Effekt zu erzielen. Die biologisch vorteilhafte Umgebung des interkorporellen Fusionsmodells konnte daher die erforderliche Menge an Wachstumsfaktoren reduzieren und deren Einsatz ökonomisch sinnvoller machen. In jedem Fall ist jedoch darauf hinzuweisen, dass die Wachstumsfaktorendosis an die biologischen Umgebungsbedingungen und den spezifischen Carrier adaptiert werden müssen. Aufgrund der geringen und lokal applizierten BMP-2 Konzentration waren in dieser Untersuchung auch keine systemischen Effekte des Wachstumsfaktors auf die Laborparameter, das Körpergewicht oder die Körpertemperatur nachweisbar.

Beim Vergleich der Wachstumsfaktorengruppen mit den Gruppen, in denen autologes Knochenmaterial verwendet wurde, zeigten sich deutliche Vorteile für die Wachstumsfaktorengruppen. Der wesentlichste Vorteil der Wachstumsfaktoren liegt zunächst darin, dass die Notwendigkeit zur Entnahme autologen Knochenmaterials entfällt, wodurch sich kausal die damit verbundene Entnahmemorbidität eliminieren läßt [11,70]. Im Vergleich zum trikortikalen autologen Beckenkammspan zeigten darüber hinaus alle Wachstumsfaktorengruppen bessere radiologische, biomechanische und histologische Fusionsparameter. Auch im Vergleich zu Cages die mit autologer Beckenkammspongiosa gefüllten waren zeigten die Wachstumsfaktoren BMP-2 und IGF-I/TGF-β1 bessere Ergebnisse hinsichtlich funktionsradiologischer Parameter, Knochendichte, Volumen des neugebildeten Kallus und biomechanischer Stabilität. Zusammenfassend läßt sich demzufolge festhalten, dass sowohl die BMP-2 als auch die IGF-I/TGF-β1 Applikation die Ergebnisse der intervertebralen Fusion mit autologem Knochenmaterial signifikant verbessern kann.

Ein weiteres Ziel dieser Untersuchungen war es, die Wirksamkeit der neuen Wachstumsfaktorenkombination IGF-I und TGF-β1 mit der etablierten Applikation

von BMP-2 zu vergleichen. Hierzu wurden äquivalente Dosen von BMP-2 bzw. IGF-I/TGF-β1 mit dem PDLLA-Carrier-System appliziert. Derzeit sind die „idealen" Dosierungen, die eine Spondylodese gewährleisten, für alle Wachstumsfaktoren unbekannt. Einige Untersuchungen in ventralen intervertebralen Fusionsmodellen [12,70,208] konnten zeigen, dass BMP-2 Konzentrationen zwischen 100 und 250 µg gute Fusionsresultate gewährleisten. In anderen Untersuchungen konnte gezeigt werden, dass ein 5:1 Verhältnis von IGF-I zu TGF-β1 am effektivsten war [95,94,97,172] und das eine Dosis von 150 µg IGF-I plus 30 µg TGF-β1 im ventralen intervertebralen Fusionsmodell der Schafhalswirbelsäule am besten zur ventralen intervertebralen Fusion geeignet war [92]. Demzufolge wurden für diesen Vergleich Konzentrationen von 150 µg BMP-2 bzw. 150 µg IGF-I plus 30 µg TGF-β1 gewählt. Bei diesen Konzentrationen konnten nur geringe und inkonstante Unterschiede zwischen den beiden Gruppen determiniert werden. Die BMP-2 Gruppe zeigte dabei eine geringere residuale Beweglichkeit auf den Funktionsröntgenbildern und eine höhere Anzahl von Versuchstieren, bei denen sich nach 9 Wochen Knochenformationen im Intervertebralraum nachweisen ließen. Die vermehrte Bildung neuer Knochenmatrix bei BMP-2 Applikation läßt sich dabei durch die spezifische Fähigkeit von BMP-2 erklären, de novo Knochen zu bilden [5,208]. Im Gegensatz dazu zeigte die IGF-I / TGF-β1-Gruppe eine höhere Knochendichte des Kallus. Auch dies läßt sich durch die spezifische Wirksamkeit von IGF-I und TGF-β1 erklären, die das Knochenremodeling durch die Beeinflussung von Osteoblasten und Osteoklasten modifizierten. Demzufolge kann festgehalten werden, dass nur geringe Unterschiede zwischen dem Fusionspotential der beiden Wachstumsfaktorengruppen in dieser Untersuchung ermittelt werden konnten. Vergleicht man aber den bisherigen experimentellen Standard BMP-2, appliziert mittels Kollagen-Carrier, mit der in dieser Untersuchung etablierten Kombination von IGF-I und TGF-β1, appliziert mittels PDLLA-Carrier, finden sich für letztgenannte Kombination signifikant bessere Fusionsresultate.

3.4 Schlußfolgerung

Ziel dieser Untersuchungen war es, den Einfluss des Cagedesigns, von Carrier-Systemen und Wachstumsfaktoren auf die intervertebrale zervikale Spondylodese am Schafsmodel zu definieren, um somit die Basis für eine optimierte Fusion der

Halswirbelsäule zu schaffen.

In diesen experimentellen Untersuchungen konnte kein direkter Zusammenhang zwischen der Primärstabilität und der Sekundärstabilität von intervertebralen Implantaten nachgewiesen werden. Daher sind rein biomechanische in vitro Untersuchungen nicht dazu geeignet das frühe Einheilungsverhalten eines Cages zu prognostizieren. Offensichtlich bestehen statt dessen „biologisch-mechanische Qualitäten“ intervertebraler Implantate, die im Cagedesign verankert sind und das Einheilungsverhalten definieren. Die Ergebnisse dieser Studien zeigen, dass die Auflagefläche für das Sinterungsverhalten eines Cages in vivo nur von untergeordneter Bedeutung ist. Hingegen stellen Designparameter wie die Größe der maximalen Pore eines Cages, die das „stress shielding“ definiert, und die Volumen-bezogene-Steifigkeit eines Cages eine „biologisch-mechanische Qualität“ dar, die eine positive Korrelation zum Einheilungsverhalten des Implantates in vivo aufweisen. Daher muß ein Umdenken in der präklinischen Evaluation von Cages, weg von rein biomechanischen in vitro Steifigkeits-Tests und hin zu tierexperimentellen in vivo Evaluationen unter Berücksichtigung von „stress-shielding“ und Volumen-bezogener-Steifigkeit gefordert werden.

In der vorliegenden Untersuchung konnte gezeigt werden, dass die biodegradierbare PDLLA-Beschichtung intervertebraler Implantate eine sichere, einfache und effektive Applikation von Wachstumsfaktoren im Intervertebralraum gewährleistet. Darüber hinaus konnte gezeigt werden, dass der PDLLA-Carrier dem Kollagen-Carrier in seiner Effektivität signifikant überlegen ist. Nebenwirkungen der PDLLA-Beschichtung konnten in dieser Untersuchung nicht ermittelt werden.

Die Applikation einer geeigneten Dosis von IGF-I und TGF-β1 mittels PDLLA beschichtetem Cage ist in der Lage, die intervertebrale Spondylodese signifikant zu stimulieren. Des weiteren konnte demonstriert werden, dass die verwendeten Wachstumsfaktoren BMP-2 bzw. IGF-I und TGF-β1 eine signifikante Verbesserung der intervertebralen Fusionsergebnisse im Vergleich zu autologem Knochenmaterial ermöglichen und gleichzeitig die Entnahme dieses Knochenmaterials überflüssig machen. Lokale oder systemische Nebenwirkungen konnten in den bisherigen Untersuchungen für die untersuchten Wachstumsfaktorendosen nicht ermittelt werden. Die in dieser Untersuchung etablierte Applikation von IGF-I und TGF-β1 mittels PDLLA-Carrier zeigte im Vergleich zum bisherigen experimentellen „golden

standard“ BMP-2 appliziert mittels Kollagen-Carrier signifikant bessere Fusionsergebnisse. Demzufolge scheint eine weitere Evaluation der kombinierten Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage gerechtfertigt.

3.5 Ausblick

3.5.1 IGF-I und TGF-β1

Alle bisherigen Ergebnisse der in vivo Untersuchungen wurden über einen Zeitraum von 12 Wochen gewonnen. Trotz sehr ermutigender Ergebnisse müssen die positiven Effekte der IGF-I und TGF-β1 Applikation mittels PDLLA-beschichtetem Cage noch in Langzeituntersuchungen bestätigt werden, bevor eine humane Anwendung dieses Verfahrens möglich ist. Zusätzlich muss die Sicherheit der IGF-I und TGF-β1 Applikation mittels PDLLA-beschichtetem Cage noch abschließend belegt werden. Obwohl in den bisherigen Untersuchungen keine negativen Effekte der Wachstumsfaktoren IGF-I und TGF-β1 oder der PDLLA-Beschichtung auf Laborparameter, Körpergewicht und Körpertemperatur nachgewiesen werden konnten, können bei systemisch wirksamen IGF-I und TGF-β1 konzentrationsabhängig unerwünschte Wirkungen auftreten. Bei systemischer Applikation von IGF-I wurden bisher neben Elektrolytverschiebungen auch Veränderungen der Serumkonzentrationen von Insulin und „growth hormon“ (GH) beschrieben. Des weiteren können Hypoglykämie, Konvulsionen, Pseudotumor cerebri, Papillenödem, Facialisparese, Parotisschwellung, Tachykardie, vorübergehend veränderte Leber-Funktions-Tests, Anti-IGF-I-Antikörper, Haarausfall und vermehrt Infektionen des oberen Respirationstraktes auftreten [62,63,195,204]. Zudem beeinflusst IGF-I die Nierenfunktion und ist bei experimentell erzeugtem Diabetes in erhöhten Konzentrationen zu finden [54,55,64,77]. Auch systemisch wirksam werdendes TGF-β1 kann negative Effekte hervorrufen. Die hochdosierte, systemische Applikation von rh-TGF-β1 erzeugt bei Ratten ein Spektrum an Läsionen in zahlreichen Zielgeweben wie Leber, Knochen, Nieren, Herz, Thymus, Pankreas, Magen und Zökum sowie im Bereich der Injektionstellen an Venen und Muskelgewebe [187]. Zusätzlich wirkt systemisch appliziertes TGF-β1 immunsuppressiv [37]. Darüber hinaus könnte die kombinierte,

lokale IGF-I und TGF-β1 Applikation noch zur Ausbildung additiver Nebenwirkungen führen, die über das Maß der Nebenwirkungen der Einzelsubstanzen hinausgehen. Um sowohl das Langzeiteinheilungsverhalten, als auch das potentielle Nebenwirkungsprofil der kombinierten, lokalen IGF-I und TGF-β1 Applikation zu evaluieren, werden daher von unseren Arbeitsgruppe derzeit entsprechende Tierversuche am zervikalen Schafsmodell durchgeführt.

3.5.2 Cages

Zunächst haben diese Untersuchungen gezeigt, dass noch ein großer Spielraum für die Optimierung des Implantatdesigns von Cages besteht. Diesbezüglich führt unsere Arbeitsgruppe derzeit weitere biomechanische Untersuchungen durch.

Unabhängig davon weist die Cage-Technologie, trotz der in diesen Untersuchungen dargelegten guten klinischen und biomechanischen Ergebnisse, aber auch Probleme auf. Die gebräuchlichsten intervertebralen Cages, einschließlich der in dieser Versuchsreihe verwendeten Cages, bestehen aus Titan-Legierungen, wodurch sich eine Beurteilung der interkorporellen Fusion im konventionellen Röntgen, CT oder MRT gelegentlich schwierig gestaltet [21,66,111]. Alternative Cagedesigne aus Karbon oder PEEK (Poly-Ether-Ether-Ketone), die eine überlagerungsfreie Bildgebung erlauben, sind metallischen Cages in Hinblick auf deren biomechanische Qualitäten unterlegen [17,100]. Außerdem existieren gegenwärtig noch keine publizierten Langzeitergebnisse intervertebraler Cages, so dass das klinische Schicksal dieser Dauerimplantate derzeit noch unklar ist. Ungeklärt, jedoch unwahrscheinlich, bleibt ebenfalls, ob langfristige toxische Effekte dieser metallischen intervertebralen Dauerimplantate, speziell unter den spezifischen Umgebungsbedingungen der Wirbelsäule, existieren.

Ein möglicher Lösungsansatz für diese Probleme stellt die Entwicklung eines biodegradierbaren intervertebralen Cages dar. Biodegradierbare Cages würden eine ungestörte Bildgebung erlauben und als Folge der Degradation nicht als Dauerimplantate im Intervertebralraum verbleiben. Zusätzlich könnten sie durch die kontinuierliche Degradation ein „stress shielding" der Implantate vermeiden. Verschiedene biodegradierbare Biomaterialien befinden sich bereits in klinischer Anwendung. Poly-(L-lactide) (PLLA), Poly-(D,L-laktide) (PDLLA) und Polyglykolide (PGA) und deren Kopolymere werden als biodegradierbare Implantate

bzw. Substanzträger in der Unfallchirurgie und Orthopädie weitverbreitet eingesetzt [41,80,82,114]. Die mechanischen Eigenschaften dieser etablierten biodegradierbaren Biomaterialien werden jedoch häufig als unzureichend für den klinischen Einsatz an der Wirbelsäule erachtet [21,66]. Die voranschreitende Entwicklung mechanisch verbesserter biodegradierbarer Composite-Materialien konnte hier jedoch Abhilfe schaffen. So konnte unsere Arbeitsgruppe analoge biomechanische Qualitäten von biodegradierbaren und metallischen Cages nachweisen [100]. Daher werden derzeit von unserer Arbeitsgruppe biodegradierbare Cages biomechanisch und speziell tierexperimentell evaluiert.

4 Zusammenfassung

Der Effekt des Cagedesigns, von Carrier-Systemen und Wachstumsfaktoren auf die intervertebrale Spondylodese am Tiermodel der Schafshalswirbelsäule wurde in einer anatomisch-biomechanischen, einer rein biomechanischen und sechs tierexperimentellen Teilstudien untersucht.

Cages weisen eine designspezifische in vitro Primärstabilität auf. Jedoch besteht kein direkter Zusammenhang zwischen der in vitro Primärstabilität und der in vivo Sekundärstabilität der Implantate. Daher sind rein biomechanische in vitro Untersuchungen nicht dazu geeignet das Einheilungsverhalten eines Cages zu prognostizieren. Die Ergebnisse dieser Studien zeigen auch, dass die Auflagefläche für das Sinterungsverhalten eines Cages in vivo nur von untergeordneter Bedeutung ist. Hingegen sind das „stress-shielding“ und die Volumen-bezogene-Steifigkeit eines Cages für das Einheilungsverhalten des Implantates in vivo entscheidend.

In den vorliegenden Untersuchungen konnte gezeigt werden, dass die biodegradierbare PDLLA-Beschichtung intervertebraler Implantate eine sichere, einfache und effektive Applikation von Wachstumsfaktoren im Intervertebralraum gewährleistet. Darüber hinaus konnte gezeigt werden, dass der PDLLA-Carrier dem Kollagen-Carrier in seiner Effektivität signifikant überlegen ist. Nebenwirkungen der PDLLA-Beschichtung konnten in diesen Untersuchungen nicht ermittelt werden.

Die Applikation einer geeigneten Dosis von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage ist in der Lage, die intervertebrale Spondylodese signifikant zu stimulieren. Des weiteren konnte demonstriert werden, dass die verwendeten Wachstumsfaktoren IGF-I und TGF-β1 eine signifikante Verbesserung der intervertebralen Fusionsergebnisse im Vergleich zu autologem Knochenmaterial ermöglichen und gleichzeitig die Entnahme dieses Knochenmaterials überflüssig machen. Die Applikation von IGF-I und TGF-β1 mittels PDLLA-Carrier zeigte außerdem im Vergleich zum bisherigen Standard BMP-2 appliziert mittels Kollagen-Carrier signifikant bessere Fusionsergebnisse. Lokale oder systemische Nebenwirkungen konnten in den bisherigen Untersuchungen für die verwendeten Wachstumsfaktorendosen nicht ermittelt werden.

Weiterführende Untersuchungen müssen belegen, ob die kombinierte lokale Applikation von IGF-I und TGF-β1 mittels PDLLA-beschichtetem Cage auch beim

Menschen zu einer signifikanten Verbesserung der Ergebnisse der zervikalen Spondylodese führen kann.

5 Literatur

1. Ahlgren BD, Vasavada MS, Brower RS, Lydon C, Herkowitz MD, Panjabi MM (1994) Annular incision technique on the strength and multidirectional flexibility of the healing intervertebral disc. Spine 19: 948-954
2. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R (2000) Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and oestrogen deficiency. J Bone Miner Res 15: 683-69
3. Aronson N, Filtzer DL, Bagan M (1968) Anterior cervical fusion by the Smith-Robinson approach. J Neurosurg 29: 397-404
4. Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA (1996) Complications of iliac crest bone graft harvesting. Clin Orthop 329: 300-309
5. Aspenberg P, Turek T (1996) BMP-2 for intramuscular bone induction. Effect of squrrel monkeys is dependent on implantation site. Acta Orthop Scand 67: 3-6
6. Bagby G (1999) The Bagby and Kuslich (BAK) method of lumbar interbody fusion. Spine 24:1857-1862
7. Bailey R, Badgley C (1960) Stabilization of cervical spine by anterior fusion. J Bone Joint Surg (Am) 42: 565-569
8. Bankwart JC, Asher MA, Hassanein RS (1995) Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. Spine 20: 1055-1060
9. Beck L, Amento E, Xu Y, Deguzman L, Lee W, Nguyen T, Gillet N (1993) TGF-beta 1 induces bone closure of skull defects – Temporal dynamics of bone formation in defects exposed to rhTGF-beta 1. J Bone Miner Res 8:753-761
10. Bent van den MJ, van Acker RE, Meyer J (1989) Anterior discectomy as treatment for a cervical radicular syndrome. Ned Tijdschr Geneeskd 133: 1550-1554
11. Boden SD, Martin GJ Jr, Horton WC, Truss TL, Sandhu HS (1998) Laproscopic anterior spinal arthrodesis with rh BMP-2 in a titanium interbody threaded cage. J Spinal Disord 11: 95-101
12. Boden SD, Martin GJ Jr, Morone MA, Ugbo JL, Moskovitz PA (1999) Posterolateral lumbar intertransverse process spine arthrodesis with recombinant human bone morphogenetic protein 2/hydroxyapatite-tricalcium phosphate after laminectomy in the nonhuman primate. Spine 24: 1179-1185
13. Boden SD, Zdeblick TA, Sandhu HS, Heim SE (2000) The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine 25: 376-381
14. Boden SD (2000) Biology of lumbar spine fusion and use of bone graft substitutes: present, future, and next generation. Tissue Eng 6: 383-399
15. Bohlmann HH, Emery SE, Goodfellow DB, Jones PK (1993) Robinson anterior cervical discectomy and arthodesis for cervical radiculopathy. J Bone Joint Surg 75-A: 1298-1307
16. Brady JM, Cutright DE, Miller RA, Barristone GC (1973) Resorption rate, route, route of elimination, and ultrastructure of the implant size of polylactic acid in the abdominal wall of the rat. J Biomed Mater Res 7:155-166
17. Brantigan JW, Steffee AD, Geiger JM (1991) A carbon fiber implant to aid interbody lumbar fusion. Mechanical testing. Spine 16: 277-282

18. Brantigan JW, Mc Afee PC, Cunningham BW (1994) Interbody lumbar fusion using a carbon fiber implant versus allograft bone. An investigational study in the Spanish goat. Spine 19:1436-1444
19. Brantigan JW, Steffee AD, Lewis ML, Quinn LM, Persenaire JM (2000) Lumbar interbody fusion using the Brantigan I/F cage for posterior lumbar interbody fusion and the variable pedicle screw placement system: two-year results from a Food and Drug Administration investigational device exemption clinical trial. Spine 25: 1437-1446
20. Brodke DS, Zdeblick TA (1992) Modified Smith-Robinson procedure for anterior cervical discetomy and fusion. Spine 17: 427-430
21. Brodke DS, Dick JC, Kunz DN, McCabe R, Zdeblick TA (1997) Posterior lumbar interbody fusion. A biomechanical comparison, including a new threaded cage. Spine 22: 26-31
22. Brooke NS, Rorke AW, King AT, Gullan RW (1997) Preliminary experience of carbon fibre cage prostheses for treatment of cervical spine disorders.Br J Neurosurg 11:221-227
23. Brown MD, Malinin TI, Davis PB (1976) A roentgenographic evaluation of frozen allografts versus autografts in anterior cervical spine fusions. Clin Orthop 119: 231-6.
24. Bubnoff von A, Cho KWY (2001) Intracellular BMP signaling regulation in vertebrates: pathway or network? Developmental Biology 239, 1-14
25. Burger EH, Klein-Nulend J, Veldhuijzen JP (1992) Mechanical stress and osteogenesis in vitro. J Bone Miner Res 7:327-401
26. Burt DW (1992) Evolutionary grouping of the transforming growth factor-beta superfamily. Biochem Biophys Res Commun. 1992 184: 590-595
27. Cain CC, Fraser RD (1995) Bony and vascular anatomy of the normal cervical spine in the sheep. Spine 20: 759-765
28. Catinella FP, De Laria GA, De Wald RL (1990) False aneurysm of the superior gluteal artery. A complication of iliac crest bone grafting. Spine 15:1360-2
29. Carter DR, Wong M (1988) Mechanical stresses and endochondral ossification in the chondroepiphysis. J Orthop Res 6:148-154
30. Centrella M, McCarthy TL, Canalis E (1989) Effects of transforming growth factors on bone cells. Connect Tissue Res 20: 267-275
31. Cloward RW (1958) The anterior approach for removal of ruptured cervical discs. J Neurosurg 15: 602-617
32. Cloward RB (1971) Complications of anterior cervical disc operation and their treatment. Surgery.69: 175-182
33. Coventry MB, Tapper EM (1972) Pelvic instability: a consequence of removing iliac bone for grafting. J Bone Joint Surg Am 54: 83-101
34. Cowley SP, Anderson LD (1983) Hernias through donor sites for iliac-bone grafts. J Bone Joint Surg Am. 65: 1023-1025
35. Colterjohn NR, Bednar DA (1997) Procurement of bone graft from the iliac crest. An operative approach with decreased morbidity. J Bone Joint Surg 79-A: 756-759
36. Cook SD, Dalton JE, Tan EH, Whitecloud TS 3rd, Rueger DC (1994) In vivo evaluation of recombinant human osteogenic protein (rhOP-1) implants as a bone graft substitute for spinal fusions. Spine 19: 1655-1663

37. Critchlow MA, Hinchliffe JR (1994) Immunolocalization of basement membrane components and beta 1 integrin in the chick wing bud identifies specialized properties of apical ectodermal ridge. Dev Biol 163: 252-269
38. Cunningham BW, Polly DW Jr. (2002) The use of interbody cage devices for spinal deformity: a biomechanical perspective. Clin Orthop 394: 73-83
39. Cunningham BW, Kanayama M, Parker LM, Weis JC, Sefter JC, Fedder IL, McAfee PC (1999) Osteogenic protein versus autologous interbody arthrodesis in the sheep thoracic spine. A comparative endoscopic study using the Bagby and Kuslich interbody fusion device. Spine 24: 509-518
40. Curylo LJ, Lindsey RW, Doherty BJ, LeBlanc A (1996) Segmental variations of bone mineral density in the cervical spine. Spine 21: 319-322
41. Cutright DE, Hunsuck EE, Beasley JD (1971) Fracture reduction using a biodegradable material, polylactic acid. J Oral Surg 29:393-397
42. David SM, Gruber HE, Mayer RA Jr, Murakami T, Tabor OB, Howard BA, Wozney JM, Hanley EN Jr (1999) Lumbar spinal fusion using recombinant human bone morphogenetic protein in the canine. A comparison of three dosages and two carriers. Spine 24: 1973-1979
43. DeBowes RM, Grant BD, Bagby GW, Gallina AM, Sande RD, Ratzlaff MH (1984) Cervical vertebral interbody fusion in the horse: a comparative study of bovine xenografts and autografts supported by stainless steel baskets. Am J Vet Res 45:191-199
44. Dennis S, Watkins R, Landaker S, Dillin W, Springer D (1989) Comparison of disc space heights after anterior lumbar interbody fusion. Spine 14: 876-878
45. De Palma AF, Rothmann LH, Lewinnek GE, Kanale TA (1972) Anterior interbody fusion for severe cervical disk degeneration. Surg Gynecol Obstet 134: 755-758
46. Ducy P, Gearard K (2000) The family of bone morphogenetic proteins. Kidney International 57, 2207-2214
47. Ebraheim NA, Rongming X, Knight T, Yeasting RA (1997) Morphometric evaluation of lower cervical pedicle and its projection. Spine 22: 1-6
48. Edmonston SJ, Singer KP, Day RE, Breidahl PD, Price RI (1994) Formalin fixation effects on vertebral bone density and failure mechnics. An study of human and sheep vertebra. Clin Biomech 9: 175-179
49. Elford PR, Lamberts SW (1990) Contrasting modulation by transforming growth factor-beta-1 of insulin-like growth factor-I production in osteoblasts and chondrocytes. Endocrinology 127: 1635-1639
50. Emery SE, Bolesta MJ, Banks MA, Jones PK (1994) Robinson anterior cervical fusion: Comparison of the standard and modified techniques. Spine 19: 660-663
51. Eysel P, Furderer S, Rompe JD, Zollner J (2000) Initial instability of different cages for fusion of the cervical spine. Zentralbl Neurochir 61: 171-176
52. Fernando TL, Kim SS, Mohler DG (1999) Complete pelvic ring failure after posterior iliac bone graft harvesting. Spine 24: 2101-2104
53. Fischgrund JS, James SB, Chabot MC, Hankin R, Herkowitz HN, Wozney JM, Shirkhoda A (1997) Augmentation of autograft using rhBMP-2 and different carrier media in the canine spinal fusion model. J Spinal Disord 10: 467-472
54. Flyvbjerg A (2001) Potential use of growth hormone receptor antagonist in the treatment of diabetic kidney disease. Growth Horm IGF Res 11: 115-119

55. Flyvbjerg A, Bennett WF, Rasch R, Kopchick JJ, Scarlett JA (1999) Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomeral hypertrophy, and urinary albumin excretion in experimental diabetes in mice. Diabetes 48: 377-382
56. Francis CC (1955) Dimensions of cervical vertebrae. Anat Rec 122: 603-609
57. Fujimoto A, Tanizawa T, Nishida S, Yamamoto N, Soshi S, Endo N, Takahashi HE (1999) Local effects of transforming growth factor-beta 1 on rat calvaria: changes depending on the dose and the injection site. J Bone Miner Metab 17: 11-17
58. Gombotz W, Pankey S, Bouchard L, Ranchalis J, Puolakkainen P (1993) Controlled release of TGF-beta 1 from a biodegradable matrix for bone regeneration. J Biomater Sci Polym Ed 5: 49-63
59. Gopferich A (1996) Mechanisms of polymer degradation and erosion. Biomaterials 17: 103-114
60. Goulet JA, Senunas LE, Desilva GL, Greenfield ML (1997) Autogenous iliac crest bone graft. Complications and functional assessment. Clin Orthop 339: 76-81
61. Grauer JN, Patel TC, Erulkar JS, Troiano NW, Panjabi MM, Friedlaender GE (2001) Evaluation of OP-1 as a graft substitute for intertransverse process lumbar fusion. Spine 26: 127-133
62. Guevara-Aguirre J, Rosenbloom AL, Vasconez O, Martinez V, Gargosky SE, Allen L, Rosenfeld RG (1997) Two-year treatment of growth hormon (GH) receptor deficiency with recombinant insulin-like growth factor I in 22 children: comparison of two dosage levels and two GH-treated GH deficiency. J Clin Endocrinol Metab 82: 629-633
63. Guevara-Aguirre J, Vasconez O, Martinez V, Martinez AL, Rosenbloom AL, Diamond FB, Gargosky SE, Nonoshita L, Rosenfeld RG (1995) A randomized, double blind, placebo-controlled trial on safety and efficacy of recombinant human insulin-like growth factor-I in children with growth hormon receptor deficiency. J Clin Endorcinol Metab 80: 1393-1398
64. Guler HP, Wettstein K, Schurr W, Zapf J, Froesch ER (1991) Recombinant human insulin-like growth factor I: effects in normal subjects and implications for use in patients. Adv Exp Med Biol 293: 97-104
65. Gunzburg R, Fraser RD, Moore R, Vernon-Roberts B (1993) An experimental study comparing percutaneous discectomy with chemonucleolysis. Spine 18: 218-226
66. Hacker RJ, Cauthen JC, Gilbert TJ, Griffith SL (2000) A prospective randomized multicenter clinical evaluation of an anterior cervical fusion cage. Spine 25: 1437-1446
67. Hacker RJ (2002) Threaded cages for degenerative cervical disease. Clin Orthop 394: 39-46
68. Harms J (2000) Interbody fusion with Meshed-Titanium-Cages. Cagemeeting 26. Oktober 2000, Hamburg
69. Hasegawa K, Abe M, Washio T, Hara T (2001) An experimental study on the interface strength between titanium mesh cage and vertebra in reference to vertebral bone mineral density. Spine 26:957-963.
70. Hecht BP, Fischgrund JS, Herkowitz HN, Penman L, Toth JM, Shirkhoda A (1999) The use of recombinant human bone morphogenetic protein 2 (rhBMP-

2) to promote spinal fusion in a nonhuman primate anterior interbody fusion model. Spine 24: 629-636
71. Heidecke V, Rainov NG, Marx T, Burkert W (2000) Outcome in Cloward anterior fusion for degenerative cervical spine disease. Acta Neurochir 142: 283-291
72. Heller JG, Zdeblick TA, Kunz DA, McCabe R, Cooke ME (1993) Spinal instrumentation for metastatic disease: in vitro biomechanical analysis. J Spinal Disord 6: 17-22
73. Hermann R, Schmidmaier G, Markl B, Resch A, Hahnel I, Stemberger A, Alt E (1999) Antithrombogenic coating of stents using a biodegradable drug delivery technology. Thromb Haemost 82: 51-57
74. Hilibrand AS, Fye MA, Emery SE, Palumbo MA, Bohlman HH (2001) Impact of smoking on the outcome of anterior cervical arthrodesis with interbody or strut-grafting. J Bone Joint Surg 83-A: 668-673
75. Hill NM, Horne JG, Devane PA (1999) Donor site morbidity in the iliac crest bone graft. Aust N Z J Surg 69: 726-728
76. Hinke V, Seck T, Clanget C, Scheidt-Nave C, Ziegler R, Pfeilschifter J (2001) Association of transforming growth factor-beta1 (TGFbeta1) T29 > C gene polymorphism with bone mineral density (BMD), changes in BMD, and serum concentrations of TGF-beta1 in a population-based sample of postmenopausal german women. Calcif Tissue Int 69: 315-320
77. Hirschberg R, Adler S (1998) Insuline-like growth factor and the kidney: Physiology, pathophysiology, and therapeutic implications. Am J Kidney Dis 31: 901-919
78. Hock J, Centrella M, Canalis E (1988) Insulin like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology 122: 254-260
79. Hollowell JP, Vollmer DG, Wilson CR, Pintar FA, Yoganandan N (1996) Biomechanical analysis of thoracolumbar interbody constructs. How important is the endplate? Spine 21: 1032-1036
80. Hollinger JO, Battistone GC (1988) Biodegradable bone repair materials. Synthetic polymers and ceramics. Clin Orthop 278:290-305
81. Hoshi K, Amizuka N, Sakou T, Kurokawa T, Ozawa H (1997) Fibroblasts of spinal ligaments pathologically differentiate into chondrocytes induced by recombinant human bone morphogenetic protein-2: morphological examinations for ossification of spinal ligaments. Bone 21: 155-162
82. Hutmacher D, Hurzeler MB, Schliephacke H (1996) A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. Int J Oral Maxillofac Implants 11: 667-678
83. Isgaard J, Nilson A, Lindahl A, Jansson J, Isaksson O (1986) Effects of local administration of GH and IGF-I on longitudinal bone growth in rats. Am J Physiol 250: 367-372
84. Itoh H, Ebara S, Kamimura M, Tateiwa Y, Kinoshita T, Yuzawa Y, Takaoka K (1999). Experimental spinal fusion with use of recombinant human bone morphogenetic protein 2. Spine 24: 1402-1405
85. Jost B, Cripton PA, Lund T, Oxland TR, Lippuner K, Jaeger P, Nolte LP (1998) Compressive strength of interbody cages in the lumbar spine: the effect of cage shape, posterior instrumentation and bone density. Eur Spine J 7: 132-141

86. Joyce ME, Roberts AB, Sporn MB, Bolander ME (1990) Transforming growth factor-beta and the initiation of chondrogenesis and osteogenesis in the rat femur. J Cell Biol 110: 2195-2207
87. Kanayama M, Cunningham BW, Haggerty CJ, Abumi K, Kaneda K, McAfee PC (2000) In vitro biomechanical investigation of the stability and stress-shielding effect of lumbar interbody fusion devices. J Neurosurg (Spine 2) 93: 259-265
88. Kandziora F, Bail H, Schmidmaier G, Schollmeier G, Scholz M, Knispel C, Pflugmacher R, Mittlmeier T, Raschke M, Haas NP (2002) BMP-2 application by a poly-(D, L-lactide) coated interbody cage: in vivo results of a new carrier for growth factors. J Neurosurg 97:40-48
89. Kandziora F, Kerschbaumer F, Starker M, Mittlmeier T (2000). Biomechanical assessment of transoral plate fixation for atlantoaxial instability. Spine 25: 1555-1561
90. Kandziora F, Kerschbaumer F, Mittelmeier T (1999) Stage related surgery for cervical spine instability in rheumatoid arthritis. Europ Spine J 8 : 371-381
91. Kandziora F, Pflugmacher R, Ludwig K, Duda G, Mittlmeier T, Haas NP (2002) Biomechanical comparison of four anterior atlantoaxial plate systems. J Neurosurg 96: 313-320
92. Kandziora F, Pflugmacher R, Scholz M, Schnake K, Schröder R, Mittlmeier T (2001) Comparison between sheep and human cervical spines: an anatomic, radiographic, bone mineral density, and biomechanical study. Spine 26: 1028-37
93. Kandziora F, Pflugmacher R, Schäfer J, Duda G, Haas NP, Mittlmeier T (2001) Biomechanical comparison of cervical spine interbody fusion cages. Spine 26: 1850-1857
94. Kandziora F, Pflugmacher R, Scholz M, Schäfer J, Schollmeier G, Schmidmaier G, Duda G, Raschke M, Haas NP (2002) Dose dependent effects of combined IGF-I and TGF-ß1 application in a sheep cervical spine interbody fusion model. Europ Spine J – published online
95. Kandziora F, Pflugmacher R, Scholz M, Knispel C, Hiller T, Schollmeier G, Bail H, Schmidmaier G, Duda G, Raschke M, Haas NP (2002) Comparison between BMP-2 and combined IGF-I/TGF-ß1 application in a sheep cervical spine fusion model. Europ Spine J 11: 482-493
96. Kandziora F, Pflugmacher R, Scholz M, Schollmeier G, Bail H, Duda G, Raschke M, Haas NP (2002) Experimentelle Spondylodese der Schafshalswirbelsäule. Teil 1: Der Effekt des Cagedesigns auf die intervertebrale Fusion. Der Chiurg 73:909-917
97. Kandziora F, Schmidmaier G, Schollmeier G, Bail H, Pflugmacher R, Görke T, Wagner M, Raschke M, Mittlmeier T, Haas NP (2002) IGF-I and TGF-ß1 application by a poly-(D,L-lactide) coated interbody cage promotes fusion in the sheep cervical spine. Spine 27:1710-1723
98. Kandziora F , Schollmeier G , Scholz M , Schaefer J , Scholz A , Schmidmaier G, Schröder R, Bail H, Duda G, Mittlmeier T, Haas NP (2002) Influence of cage design on interbody fusion in a sheep cervical spine model. J Neurosurg 96; 321-332
99. Kandziora F, Scholz M, Pflugmacher R, Krummrey G, Schollmeier G, Schmidmaier G, Schnake KJ, Duda G, Raschke M, Haas NP (2002) Experimentelle Spondylodese der Schafshalswirbelsäule. Teil 2: Der Effekt

von Carrier-Systemen und Wachstumsfaktoren auf die intervertebrale Fusion. Der Chiurg – im Druck
100. Kandziora F, Pflugmacher R, Kleemann R, Duda G, Wise DL, Trantolo DJ, Lewandrowski KU (2002) Biomechanical analysis of biodegradable interbody fusion cages augmented with poly(propylene glycol-co-fumaric acid). Spine 27:1644-51
101. Karaikovic EE, Daubs MD, Madsen RW, Gaines RW (1997) Morphometric characteristics of human cervical pedicles. Spine 22: 493-500
102. Kettler A, Wilke HJ, Dietl R, Krammer M, Lumenta C, Claes L (2000) Stabilising effect of posterior lumbar interbody fusion cages before and after cyclic loading. J Neurosurg 92: 87-92
103. Kim Y (2001) Prediction of mechanical behaviors at interfaces between bone and two interbody cages of lumbar spine segments. Spine.26: 1437-1442
104. Kleemann R (1999) Entwicklung eines Wirbelsäulenprüfstands zur Testung von Implantaten an der Halswirbelsäule; Semesterarbeit. Fakultät für Maschinenbau. Technische Universität Berlin
105. Kleeman TJ, Ahn UM, Talbot-Kleeman A (2001) Laparoscopic anterior lumbar interbody fusion with rhBMP-2: a prospective study of clinical and radiographic outcomes. Spine 26: 2751-2756
106. Kon T, Yamazaki M, Tagawa M, Goto S, Terakado A, Moriya H, Fujimura S (1997) Bone morphogenetic protei-2 stimulates differentiation of cultured spinal ligament cells from patients with ossification of the posterior longitudinal ligament. Calcif Tissue Int 60: 291-296
107. Kulkarni RK, Moore EG, Hegyeli AF, Leonard F (1971) Biodegradable poly(lactic acid) polymers. J Biomed Mater Res 5: 169-181
108. Kumar A, Kozak JA, Doherty BJ, Dickson JH (1993) Interspace distraction and graft subsidence after anterior lumbar fusion with femoral strut allograft. Spine 18:2393-2400
109. Kumta SM, Spinner R, Leung PC (1992) Absorbable intramedullary implants for hand fractures. Animal experiment and clinical trial. J Bone Joint Surg 93-B: 839-843
110. Kuslich SD, Ulstrom CL, Griffith SL, Ahern JW, Dowdle JD (1998) The Bagby and Kuslich method of lumbar interbody fusion. History, techniques, and 2-year follow-up results of a United States prospective, multicenter trial. Spine 23: 1267-1278
111. Kuslich SD, Danielson G, Dowdle JD, Sherman J, Fredrickson B, Yuan H, Griffith SL (2000) Four-year follow-up results of lumbar spine arthrodesis using the Bagby and Kuslich lumbar fusion cage. Spine 25: 2656-2662
112. Laing RJ, Ng I, Seeley HM, Hutchinson PJ (2001) Prospective study of clinical and radiological outcome after anterior cervical discectomy. Br J Neurosurg 15: 319-323
113. Lange M, Philipp A, Fink U, Oeckler R (2000) Anterior cervical spine fusion using RABEA-Titan-cages avoiding iliac crest spongiosa: first experience and results. Neurol Neurochir Pol 34: 64-69
114. Laurencin C, Lane JM (1994) Poly (lactide acid) and Poly (glycolid acid): Orthopedic surgery applications. In: Bone formation and repair. Brighton C, Frielaender G, Lane MJ. (eds.) Rosemont, American Academy of Orthopedic Surgeons, pp 325-339

115. Laursen M, Hoy K, Hansen ES, Gelineck J, Christensen FB, Bunger CE (1999) Recombinant bone morphogenetic protein-7 as an intracorporal bone growth stimulator in unstable thoracolumbar burst fractures in humans: preliminary results. Eur Spine J 8: 485-490
116. Lee EJ, Hung YC, Lee MY, Yan JJ, Lee YT, Chang JH, Chang GL, Chung KC (1999) Kinematics of cervical spine discectomy with and without bone grafting: quantitative evaluation of late fusion in a sheep model. Neurosurgery 44: 139-146
117. Lee SW, Lim TH, You JW, An HS (2000) Biomechanical effects of anterior grafting devices on the rotational stability of spinal constructs. J Spinal Disord 13:150-155
118. Lind M (1998) Growth factor stimulation on bone healing. Effects on osteoblasts, osteotomies, and implants fixations. Acta Orthop Scand Suppl 283: 2-37
119. Lind M, Schuhmacker B, Soballe K, Keller J, Melson F, Bunger C (1993) Transforming growth factor-beta enhances fracture healing in rabbit tibiae. Act Orthop Scand 64: 553-556
120. Linkhart TA, Keffer MJ (1991) Differential regulation of insulin-like growth factor-I (IGF-I) and IGF-II release from cultured neonatal mouse calvaria by parathyroid hormone, transforming growth factor-beta, and 1,25-dihydroxyvitamin D3. Endocrinology 128: 1511-1518
121. Linkhart TA, Mohan S, Baylink DJ (1996) Growth factors for bone growth and repair: IGF, TGF beta and BMP. Bone 19: 1-12
122. Liu P, Oyajobi BO, Russell RG, Scutt A (1999) Regulation of osteogenic differentiation of human bone marrow stromal cells: interaction between transforming growth factor-beta and 1,25(OH)(2) vitamin D(3) In vitro. Calcif Tissue Int 65: 173-180
123. Lund T, Oxland TR, Jost B, Cripton P, Grassmann S, Etter C, Nolte LP (1998) Interbody cage stabilisation in the lumbar spine: biomechanical evaluation of cage design, posterior instrumentation and bone density. J Bone Joint Surg Br 80: 351-359
124. Ma ZJ, Misawa H, Yamaguchi M (2001) Stimulatory effect of zinc on insuline-like growth factor I and transforming growth factor-beta 1 production with bone growth of newborn rats. Int J Mol Med 8: 623-628
125. Madawi AA, Powell M, Crockard HA (1996) Biocompatible osteoconductive polymer versus iliac graft. A prospective comparative study for the evaluation of fusion pattern after anterior cervical discectomy. Spine 21: 2123-2139
126. Magin MN, Delling G. Improved lumbar vertebral interbody fusion using rhOP-1: a comparison of autogenous bone graft, bovine hydroxylapatite (Bio-Oss), and BMP-7 (rhOP-1) in sheep. Spine 26:469-478
127. Martin GJ Jr, Boden SD, Marone MA, Marone MA, Moskovitz PA (1999) Posterolateral intertransverse process spinal arthrodesis with rhBMP-2 in a nonhuman primate: important lessons learned regarding dose, carrier, and safety. J Spinal Disord 12: 179-186
128. Majola A, Vainionpaa S, Vihtonen K, Mero M, Vasenius J, Tormala P, Rokkanen P (1991) Absorption, biocompatibility and fixation properties of polylactic acid in bone tissue: an experimental study in rats. Clin Orthop 244: 260-269
129. McClure P, Siegler S, Nobilini R (1998) Three dimensional flexibility characteristics of the human cervical spine in vivo. Spine 23: 216-223

130. Melrose J, Ghosh P, Taylor TKF, Hall A, Osti OL, Vernon-Roberts B, Fraser RD (1992) A longitudinal study of the matrix changes induced in the intervertebral discs by surgical damage to the annulus fibrosus. J Orthop Res 10: 665-676
131. Meyer RA Jr, Gruber HE, Howard BA, Tabor OB Jr, Murakami T, Kwiatkowski TC, Wozney JM, Hanley EN Jr (1999) Safety of recombinant human bone morphogenetic protein-2 after spinal laminectomy in the dog. Spine 24: 747-754
132. Miller RA, Brady JM, Cutright DE (1977) Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. J Biomed Mater Res 11: 711-719
133. Mimatsu K, Kishi S, Hashizume Y (1997) Experimental chronic compression on the spinal cord of the rabbit by ectopic bone formation in the ligamantum flavum with bone morphogenetic protein. Spinal Cord 35: 740-746
134. Mohan S, Baylink D. Bone growth factors (1991) Clin Orthop Rel Res 263: 30-48
135. Moore RJ, Osti OL, Vernon-Roberts B, Fraser RD (1992) Changes in endplate vascularity after an outer annulus tear in the sheep. Spine 17: 874-878
136. Nibu K, Panjabi MM, Oxland T, Cholewicki J (1997) Multidirectional stabilising potential of BAK interbody spinal fusion system for anterior surgery. J Spinal Disord 10: 357-362
137. Nielson H, Isgaard J, Lindahl A, Peterson L, Isaksson O (1987) Effects of unilateral arterial infusion of GH and IGF-I on tibial longitudinal bone growth in hypophysectomized rats. Calcif tissue Int 40: 91-96
138. Onari K, Akiyama N, Kondo S, Toguchi A, Mihara H, Tsuchiya T (2001) Long-term follow-up results of anterior interbody fusion applied for cervical myelopathy due to ossification of the posterior longitudinal ligament. Spine 26: 488-493.
139. Osti OL, Vernon-Roberts B, Fraser RD (1990) Annulus tear an intervertebral disc degeneration: An experimental study using an animal model. Spine 15: 431-435
140. Oxland TR, Hoffer Z, Nydegger T, Rathonyi GC, Nolte LP (2000) A comparative biomechanical investigation of anterior lumbar interbody cages: central and bilateral approches. J Bone Joint Surg 82-A: 383-393
141. Ozuna R, Sandhu HS, Kanim LEA (1995) Carriers of bone morphogenetic protein for posterolateral spinal fusion. Proccedings of the North American Spine Society 10th Annual Meeting, p 14
142. Paar O, Andereya S, Staatz G, Ambacher T, Erli HJ (2001)Value of human recombinant osteogenetic proteins as bone replacement materials in lumbar spondylodesis. Results of an animal experiment study. Unfallchirurg 104: 700-709
143. Paramore CG, Lauryssen C, Rauzzino MJ, Wadlington VR, Palmer CA, Brix A, Cartner SC, Hadley MN (1999) The safety of OP-1 for lumbar fusion with decompression - a canine study. Neurosurgery 44:1151-1155
144. Parthiban JK, Singhania BK, Ramani PS. A radiological evaluation of allografts (ethylene oxide sterilized cadaver bone) and autografts in anterior cervical fusion. Neurol India 50: 17-22

145. Patel TC, Erulkar JS, Grauer JN, Troiano NW, Panjabi MM, Friedlaender GE (2001) Osteogenic protein-1 overcomes the inhibitory effect of nicotine on posterolateral lumbar fusion. Spine 26: 1656-1661
146. Pettersson K, Hildingsson C, Fagerlund MBJ (1997) Disc pathology after whiplash injury. Spine 22: 283-288
147. Pfeilschifter J, Oechsner M, Naumann A, Gronwald R, Minne H, Ziegler R (1990) Stimulation of bone matrix apposition in vitro by local growth factors: a comparison between insulin- like growth factor I, platelet derived growth factor and transforming growth factor beta. Endocrinology 127: 69-75
148. Pfeilschifter J, Erdmann J, Storch S, Ziegler R, Weinreb M (1999) Changes in the concentration of insulin-like growth factor I and transforming growth factor beta1 in rat femoral bone during growth. Calcif Tissue Int 64: 78-82
149. Pfeilschifter J, Laukhuf F, Muller-Beckmann B, Blum WF, Pfister T, Ziegler R (1995) Parathyroid hormone increases the concentration of insulin-like growth factor-I and transforming growth factor beta 1 in rat bone. J Clin Invest 96: 767-774
150. Porchet F, Jaques B (1996) Unusual complications at iliac crest bone graft donor site: experience with two cases. Neurosurgery 39: 856-859
151. Pralkar VM, Grasser WA, Mansolf AL, Baumann AP, Owen TA, Smock SL, Martinovic S, Borovecki F, Vukicevic S, Ke HZ, Thompson DD (2002) Regulation of BMP-7 expression by retinoic acid and prostaglandin E(2). J Cell Physiol 190: 207-217
152. Profeta G, de Falco R, Ianniciello G, Prfeta L, Cigliano A, Raja A (2000) Preliminary experience with anterior cervical microdiscectomy and anterior titanium cage fusion (Novus CT-Ti) in patients with cervical disc disease. Surg Neurol 53: 417-426
153. Rapoff AJ, Ghanayem AJ, Zdeblick TA (1997) Biomechanical comparison of posterior lumbar interbody fusion cages. Spine 22: 2375-2379
154. Raschke M, Wildemann B, Inden P, Bail H, Flyvbjerg A, Hoffmann J, Haas NP, Schmidmaier G (2002) Insulin-like growth factor-1 and transforming growth factor beta 1 accelarates osteotomy healing using polylactide coated implants as a delivery sysrtem: a biomechanical and histological study in minipigs. Bone 30: 144-151
155. Riew KD, Rhee JM (2002) The use of titanium mesh cages in the cervical spine. Clin Orthop 394: 47-54
156. Rinderknecht E, Humbel RE (1978) The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J Biol Chem 253: 2769-2776
157. Roberts A, Sporn M, Bolander M (1990) Transforming growth factor-beta and the initiation of chondrogenesis in the rat femur. J Cell Biol 110: 2195-2207
158. Robinson RA, Walker AE, Ferlic DC, Wiecking DK (1962) The results of anterior interbody fusion of the cervical spine. J Bone Joint Surg 44-A: 1569-1587
159. Robinson R, Smith G (1955) Antero-lateral cervical disc removal and interbody fusion for cervical disc syndrome. Bull John Hopkins Hosp 96: 223-224
160. Robey PG, Young MF, Flanders KC, Roche NS, Kondaiah P, Reddi AH, Termine JD, Sporn MB, Roberts AB (1987) Osteoblasts synthesize and

respond to transforming growth factor-type beta (TGF-beta) in vitro. J Cell Biol 105: 457-463
161. Saitoh H, Takata T, Nikai H, Shintani H, Hyon SH, Ikada Y (1994) Effect of polylactic acid on osteoinduction of demineralized bone: preliminary study of the usefulness of polylactic acid as a carrier of bone morphogenetic protein. J Oral Rehabil 21:431-438
162. Sandhu HS, Khan SN, Suh DY, Boden SD (2001) Demineralized bone matrix, bone morphogenetic proteins, and animal models of spine fusion: an overview. Eur Spine J (10 Suppl) 2: 122-131.
163. Sandhu HS, Kanim LE, Kabo JM, Toth JM, Zeegen EN, Liu D, Delamater RB, Dawson EG (1996) Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion. Spine 21: 2115-22
164. Sandhu HS, Kanim LE, Toth JM, Kabo Jm, Liu D, Delamarter RB, Dawson EG (1997) Experimental spinal fusion with recombinant human bone morphogenetic protein-2 without decortication of osseous elements. Spine 22: 1171-1180
165. Sandhu HS, Turner S, Kabo M, Kanim LE, Liu D, Nourparvar A, Delamater RB, Dawson EG (1996) Distractive properties of threaded interbody fusion device. An in vivo model. Spine 21:1201-1210
166. Sandhu HS, Toth JM, Diwan AD, Seim HB 3rd, Kanim LE, Kabo JM, Turner AS (2002) Histologic evaluation of the efficacy of rhBMP-2 compared with autograft bone in sheep spinal anterior interbody fusion. Spine 27: 567-575
167. Sano A, Hojo T, Maeda M, Fujioka K (1998) Protein release from collagen matrices. Adv Drug Deliv Rev 31: 247-266
168. Savolainen S, Usenius JP, Hernesniemi J (1994) Iliac crest versus artificial bone grafts in 250 cervical fusions. Acta Neurochir 129: 54-57
169. Sawin PD, Traynelis VC, Menezes AH (1998) A comparative analysis of fusion rates and donor-site morbidity for autogeneic rib and iliac crest bone grafts in posterior cervical fusions. J Neurosurg 88: 255-265
170. Scheven BA, Hamilton NJ, Fakkeldij TM, Duursma SA (1991) Effects of recombinant human insulin-like growth factor I and II (IGF-I/-II) and growth hormone (GH) on the growth of normal adult human osteoblast-like cells and human osteogenic sarcoma cells. Growth Regul 1: 160-167
171. Schmidmaier G, Wildemann B, Stemberger A, Haas NP, Raschke M (2001) Biodegradable poly-(D,L-lactide) coating of implants for contionous release of growth factors. J Biomed Mat Res 58: 449-455
172. Schmidmaier G, Wildemann B, Stemberger A, Haas NP, Raschke M (2001) Local application of growth factors (insulin-like growth factor-1 and transforming growth factor-beta1) from a biodegradable poly(D,L-lactide) coating of osteosynthetic implants accelerates fracture healing in rats. Bone 28: 341-350
173. Schnee CL, Freese A, Weil RJ, Marcotte PJ (1997) Analysis of harvest morbidity and radiographic outcome using autograft for anterior cervical fusion. Spine 22: 2222-2227
174. Schroder J, Wassmann H (2001) Polymethylmethacrylate (PMMA) in anterior cervical spine surgery - current situation in Germany. Zentralbl Neurochir 62: 33-36

175. Shimamoto N, Cunningham BW, Dmitriev AE, Minami A, McAfee PC (2001) Biomechanical evaluation of stand-alone interbody fusion cages in the cervical spine. Spine 26: 432-436
176. Shono Y, Mc Afee PC, Cunningham BW, Brantigam JW (1993) A biomechanical analysis of decompression and reconstruction methods in the cervical spine. Emphasis on a carbon-fiber-composite cage. J Bone Joint Surg 75-A: 1674-1684
177. Slappey G, Toribatake Y, Ganey TM, Odgen JA, Hutton WC (1998) Guidelines to decortication in posterolateral spine fusion. J Spinal Disord 11: 102-109
178. Slater R, Nagel R, Smith RL (1998) Biochemistry of fusion mass consolidation in the sheep spine. J Ortho Res 6: 138-144
179. Smit TH (2002) The use of a quadruped as an in vivo model for the study of the spine - biomechanical considerations. Eur Spine J 11: 137-44
180. Sorensen TS, Sorensen AI, Merser S (1990) Rapid release of gentamicin from collagen sponge. In vitro comparison with plastic beads. Acta Orthop Scand 61: 353-356
181. Steinbrech DS, Mehrara BJ, Rowe NM, Dudziak ME, Luchs JS, Saadeh PB, Gittes GK, Longaker MT (2000). Gene expression of TGF-beta, TGF-beta receptor, and extracellular matrix proteins during membranous bone healing in rats. Plast Reconstr Surg 105: 2028-38
182. Suh DY, Boden SD, Louis-Ugbo J, Mayr M, Murakami H, Kim HS, Minamide A, Hutton WC (2002) Delivery of recombinant human bone morphogenetic protein-2 using a compression-resistant matrix in posterolateral spine fusion in the rabbit and in the non-human primate. Spine 27: 353-360
183. Summers BN, Eisenstein SM (1989) Donor site pain from the ilium. A complication of lumbar spine fusion. J Bone Joint Surg 71-B: 677-680
184. Takaoka K, Koezuka M, Nakahara H (1991) Telepeptide-depleted bovine skin collagen as a carrier for bone morphogenetic protein. J Orthop Res 9: 902-907
185. Tamada JA, Langer R (1993) Erosion kinetics of hydrolytically degradable polymers. Proc Natl Acad Sci USA 90:552-556
186. Tencer AF, Hampton D, Eddy S (1995) Biomechanical properties of threaded inserts for lumbar interbody spinal fusion. Spine 20: 2408-2414
187. Terrell TG, Working PK, Chow CP, Green JD (1993) Pathology of recombinant human transforming growth factor-beta 1 in rats and rabbits. Int Rev Exp Pathol 34B: 43-67
188. Thaller S, Hoyt J, Tesluck H, Holmes R (1993) The effect of insulin growth factor – I on calvarial sutures in Sprague-Dawley rat. J Craniofac Surg 4: 35-39
189. Thorell W, Cooper J, Hellbusch L, Leibrock L (1998) The long-term clinical outcome of patients undergoing anterior cervical discectomy with and without intervertebral bone graft placement. Neurosurgery 43: 268-273
190. Tremollieres FA, Strong DD, Baylink DJ, Mohan S (1991) Insulin-like growth factor II and transforming growth factor beta 1 regulate insulin-like growth factor I secretion in mouse bone cells. Acta Endocrinol 125: 538-546
191. Trippel S, Coutts R, Einhorn T, Mundy R, Rosenfeld R (1996) Growth factors as therapeutic agents. J Bone Joint Surg 78-A: 1272-1286
192. Urist MR (1965) Bone: formation by autoinduction. Science 150: 893-899
193. Vaccaro AR, Cirello J (2002) The use of allograft bone and cages in fractures of the cervical, thoracic, and lumbar spine. Clin Orthop 394: 19-26

194. VandePol C, Schlaeger D, Wong GL (1989) Mitogenic responses to and binding of insulin-like growth factor 1 and/or epidermal growth factor by bone cells. Bone Miner 5: 371-382
195. Vasconez O, Martinez V, Martinez AL, Hidalgo F, Diamond FB, Rosenbloom AL, Rosenfeld RG, Guevara-Auguirre J (1994) Heart rate increases in patient with growth hormone receptor deficiency treated with insulin-like growth factor I. Acta Paediatr Suppl 399: 137-139
196. Vetter U, Zapf J, Heit W, Helbing G, Heinze E, Froesch ER, Teller WM (1986) Human fetal and adult chondrocytes. Effect of insulinlike growth factors I and II, insulin, and growth hormone on clonal growth. J Clin Invest 77: 1903-1908
197. Villas C, martinez-Peric R, Preite R, Barrios RH (1994) Union after multiple cervical spine fusion. 21 cases followed for 1-6 years. Acta Orthop Scand 65: 620-622
198. Volkman T, Horton WC, Hutton WC (1996) Transfacet screws with lumbar interbody reconstruction: biomechanical study of motion segment stiffness. J Spinal Disord 9: 425-432
199. Weber FE, Eyrich G, Gratz KW, Thomas RM, Maly FE, Sailer HF (2001) Disulfide bridge conformers of mature BMP are inhibitors for heterotopic ossification. Biochem Biophys Res Commun 286: 554-558
200. Weiner BK, Fraser RD (1998) Spine update. Lumbar interbody cages. Spine 23: 634-640
201. Wilke HJ, Kettler A, Claes LE (1997) Are sheep spines a valid biomechanical model for human spines? Spine 22: 2365-2374
202. Wilke HJ, Kettler A, Wenger KH, Claes LE (1997) Anatomy of the sheep spine and its comparison to the human spine. Ana Rec 247: 542-555
203. Wilke HJ, Kettler A, Claes L (2000) Primary stabilizing effect of interbody fusion devices for the cervical spine: an in vitro comparison between three different cage types and bone cement. Eur Spine J 9: 410-416
204. Wilton P (1992) Treatment with recombinant human insulin-like growth factor I of children with growth hormone receptor deficiency (Laron syndrome). Kabi Pharmacia Study Group on insulin-like growth factor I treatment in growth hormone insensitivity syndromes. Acta Paediatr Suppl 383: 137-142
205. Wood GW 2nd, Boyd RJ, Carothers TA, Mansfield FL, Rechtine GR, Rozen MJ, Sutterlin CE 3rd (1995) The effect of pedicle screw/plate fixation on lumbar/lumbosacral autogenous bone graft fusions in patients with degenerative disc disease. Spine 20: 819-830
206. Yamada Y, Harada A, Hosoi T, Miyauchi A, Ikeda K, Otha H, Shiraki M (2000) Association of transforming growth factor beta 1 genotype with therapeutic response to active vitamin D for postmenopausal osteoporosis. J Bone Miner Res 15: 415-420
207. Yamamuro T, Shikata J, Okumura H, Kitsugi T, Kakutani Y, Matsui T, Kokubo T (1990) Replacement of the lumbar vertebrae of sheep with ceramic prostheses. J Bone Joint Surg 72-B: 889-893
208. Zdeblick TS, Ganayem AJ, Rapoff AJ, Swain C, Bassett T, Cooke ME, Markel M (1998) Cervical interbody fusion cages. An animal model with and without bone morphogenetic protein. Spine 23: 758-765
209. Zeidman SM, Ducker TB, Raycroft J (1997) Trends and complications in cervical spine surgery: 1989-1993. J Spinal Disord 10: 523-526

210. Zegzula HD, Buck DC, Brekke J, Wozney JM, Hollinger JO (1997) Bone formation with use of rhBMP-2 (recombinant human bone morphogenetic protein-2). J Bone Joint Surg 79-A: 1778-1790

6 Anhang

6.1 Verzeichnis der Abkürzungen

BAK	Bagby and Kuslich
BMP-2	Bone Morphogenetic Protein - 2
BMP-7	Bone Morphogenetic Protein - 7
C3/4	Intervertebralraum zwischen Halswirbelkörper 3 und 4
CT	Computertomographie
GH	growth hormone
IGF-I	Insulin Like Growth Factor - I
MRT	Magnetresonanztomographie
OPLL	Ossification of the Posterior Longitudinal Ligament
OP-1	Osteogenetic Protein – 1 (Synonym für BMP-7)
PDLLA	Poly (D,L-laktid)
PEEK	Poly-Ether-Ether-Keton
PGA	Poly-Glykolidsäuren
PLA	Poly-Laktidsäuren
rh	rekombinant human
TGF-β1	Transforming Growth Factor beta - 1
µg	Mikrogramm

6.2 Begriffsdefinition

Cage	interkorporelles Wirbelsäulenimplantat
Carrier	Trägermaterial zur Applikation biologisch aktiver Substanzen
Harms-Cage	interkorporelles zylindrisches Wirbelsäulenimplantat (Meshed Titanium Cage) nach Harms
PDLLA-Carrier	Trägermaterial aus Poly (D,L-laktid)
Primärstabilität	Biomechanische Steifigkeit eines Bewegungssegmentes direkt nach Implantation des Implantats
Sekundärstabilität	Biomechanische Steifigkeit eines Bewegungssegmentes nach zeitlich begrenzter Einheilung des Implantates
Spondylodese	Fusion eines Bewegungssegmentes der Wirbelsäule
Steifigkeit	biomechanischer Parameter: beschreibt das einwirkende Moment (Nm) in Relation zum Bewegungsausmaß (Grad)
Stress-Shielding	Protektion vor der Einwirkung mechanischer Kräfte
Syncage-C	interkorporelles box-förmiges Halswirbelsäulenimplantat
Wachstumsfaktoren	niedermolekulare lösliche Proteine mit biologischer Funktion

Danksagung

Zunächst gilt mein ganz besonderer Dank meinem Chef, **Professor Dr. Norbert P. Haas**, für die mir seinerseits gewährten Freiräume, ohne die die zeitraubenden experimentellen Untersuchungen nicht möglich gewesen wären. Zusätzlich danke ich ihm für seine vielfältige Unterstützung, sowie für die Möglichkeit und das Vertrauen, die gewonnenen Erkenntnisse in den klinischen Alltag umsetzen zu können.

Mein ganz besonderer Dank gilt auch **Professor Dr. Thomas Mittlmeier**, Ordinarius an der Universität Rostock, für die klinische Umsetzung der gewonnenen Erkenntnisse in die konkrete Behandlung unserer Patienten. Des weiteren danke ich ihm für die geduldige Assistenz bei meiner ersten Halswirbelsäulenoperation.

Herrn **Professor Dr. Fridun Kerschbaumer**, Leiter der Klinik für Rheumaorthopädie an der Orthopädischen Universitätklinik Frankfurt, möchte ich von Herzen für die Starthilfe und Rückendeckung bei meinem Einstieg in das eigenständige wissenschaftliche Arbeiten danken.

Zutiefst möchte ich auch **Professor Dr. Jürgen Harms**, Karlsbad, danken, der mir mit seinen sehr innovativen und kreativen Ideen und seiner Wertschätzung den Boden für die hier präsentierten wissenschaftlichen Leistungen bereitet hat.

Danken möchte ich ebenso meinen Kollegen/innen **Robert Pflugmacher** und **Dr. Georg Schollmeier**, Potsdam, für die unermüdliche und tatkräftige Unterstützung bei der Durchführung der tierexperimentellen Untersuchungen. Des weiteren danke ich meinen Kollegen **Dr. Hermann-J. Bail**, **Dr. Gerhard Schmidmaier**, **Dr. Klaus J. Schnake**, **Dr. Cyrus Khodadadyan-Klostermann**, **Priv.-Doz. Dr. Andreas Weiler** und **Priv.-Doz. Dr. Michael J. Raschke** für die jederzeit gewährte konstruktive Diskussionszeit und mannigfaltige Unterstützung.

Mein Dank gilt ebenso den Mitgliedern der Cervical Spine Expert Group der AO, **Kai-Uwe Lewandrowski**, M.D., Boston, USA und **Prof. Dr. Donald Wise**, Boston, USA für den wissenschaftlichen Gedankenaustausch.

Vielen herzlichen Dank an unsere ehemaligen und noch tätigen Doktoranden, **Matti Scholz** (Unfallchirurgie, Charité, Berlin), **Jan Schäfer** (Unfallchirurgie, Charité, Berlin), **Kathrin Ludwig** (Unfallchirurgie, Charité, Berlin), **Thomas Görke** (Neurochirurgie, Bern, Schweiz), **Alexandra Scholz**, **Martin Wagner**, **Tino Hiller**, **Christian Knispel**, **Niels Schulze-Stahl** und **Tanja Eindorf** (Tierärztin,

Unfallchirurgische Forschung, Charité) für die Unmengen an Zeit und die Leidenschaft, die sie in die Durchführung der experimentellen Untersuchungen investiert haben.

Ebenso danke ich den Mitarbeitern unserer Forschungseinrichtung, **Frau Marzena Princ**, **Frau Gabriele Hardung**, **Frau Dr. Brit Wildemann**, **Herrn Dipl. Ing. Pierre Kassi**, **Herrn Dipl. Ing. Jan-H. Hoffmann** und **Herrn Professor Dr. Ing. Georg Duda**, sowie **Herrn Kahled Hazim** und **Herrn Priv.-Doz. Dr. Ralf Schröder** aus der Radiologischen Klinik des Hauses, für die jederzeit gewährte tatkräftige Unterstützung bei der Umsetzung der Versuche.

Ohne die finanzielle Unterstützung durch Hr. Lutz Biedermann über das Max-Biedermann Institut der Steinbeis Stiftung, die Deutsche Gesselschaft für Unfallchirurgie, die Deutsche Forschungsgemeinschaft, die Forschungskommission der Charité und die Firmen Mathys, Bettlach, Schweiz, Cambridge Sientific Inc., Bosten, USA, Aesculap, Tuttlingen, Biomet Merck, Berlin und Karl Storz GmbH, Tuttlingen, wäre die Durchführung der Experimente nicht möglich gewesen. Hierfür möchte ich mich herzlich bedanken.

Eidesstattliche Versicherung

gemäß der Habilitationsordnung der Charité

Hiermit erkläre ich, dass

- keine staatsanwaltlichen Ermittlungsverfahrengegen mich anhängig sind,

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,

- die vorglegte Habilitationsschrift ohne fremde Hilfe verfaßt, die beschriebenen Ergebnisse selbst gewonnen wurden, sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlerinnen oder Wissenschaftlern und technischen Hilfskräften und die Literatur vollständig angegeben sind,

- mir die geltende Habilitationsordnung bekannt ist.

Berlin, den 14.02.03

Dr. med. Frank Kandziora